

# abstract book



## **Society of Environmental Toxicology and Chemistry North America 33<sup>rd</sup> Annual Meeting**

Catching the Next Wave: Advancing Science Through  
Innovation and Collaboration

Long Beach Convention & Entertainment Center, Long Beach, California  
11–15 November 2012



# Abstract Book

SETAC North America 33<sup>rd</sup> Annual Meeting

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This book comprises the abstracts of the presentations for the platform and poster sessions of the 33<sup>rd</sup> Annual Meeting in North America of the Society of Environmental Toxicology and Chemistry (SETAC), conducted at the Long Beach Convention & Entertainment Center in Long Beach, California, 11–15 November 2012. The abstracts are reproduced as accepted by the Scientific Program Committee and appear in numerical order. In each abstract, the presenting author's name is underlined.

The author index cross-references the corresponding abstract numbers. Affiliation, session and keyword indices are also included.

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International Standard Serial Number 1087-8939

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The Society of Environmental Toxicology and Chemistry (SETAC), with offices in North America and Europe, is a nonprofit, professional society established to provide a forum for individuals and institutions engaged in the study of environmental problems, management and regulation of natural resources, education, research and development, and manufacturing and distribution.

Specific goals of the society are:

- Promote research, education, and training in the environmental sciences
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- Participate in the scientific interpretation of issues concerned with hazard assessment and risk analysis
- Support the development of ecologically acceptable practices and principles
- Provide a forum (meetings and publications) for communication among professionals in government, business, academia, and other segments of society involved in the use, protection, and management of our environment

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**Environmental Quality Through Science®**



**1 Biodegradation of MC252 Oil in Marsh Sediments** R. Atlas, University of Louisville / Dept. of Biology; S. Faith, Battelle; L. Cook, Exponent; K. Murray, Exponent; K. Cerrito, Exponent, Inc.; P.D. Boehm, Exponent, Inc. / Environmental & EcoScience Group. Biodegradation of MC252 oil in marsh sediments that had varying degrees of oiling was examined at 27 sites along the Louisiana coast. Surface samples (0-2 cm) were collected in Fall 2010 and sediment cores were collected at varying distances from the shorelines in Fall 2011. The sediment cores were sectioned and aliquots at different depths were taken for chemical and microbiological (molecular) analyses. Microbial analyses using PhyloChip showed significant differences between *Spartina*, mixed mainland, and mangrove plant habitats regardless of the degree of oiling. Oil penetrated below 4 cm at eight sites with penetration to >8 cm in three cores. Compared to the oil released at the wellhead, the oil in the shoreline sediments was highly degraded as evidenced by the extensive loss (>90%) of alkanes and polycyclic aromatic hydrocarbons in the majority of the MC252 oiled sediment samples. Changes in C17/pristane, C18/phytane, C2phenanthrene/C2dibenzothiophene, C3phenanthrene/C3dibenzothiophene, total polycyclic aromatics to hopane, and total heavy polycyclic aromatics (4-6 rings) to hopane in samples having sufficient oil for analysis, showed evidence for extensive biodegradation as well as other weathering processes that had affected the oil as it moved to shore. In the cores collected in Fall 2011 there were additional losses of PAH and further changes in the ratios of alkylated phenanthrenes to alkylated dibenzothiophenes compared to surface shoreline samples that had been collected in 2010 at some of the sites, indicating that biodegradation was continuing in the sediments. Molecular analyses performed with GeoChip and high-throughput whole metagenomic sequencing indicated increased abundances of genes involved in alkane, aromatic, and cycloparaffinic hydrocarbons within oiled samples when stratified by vegetation type.

**2 Bioavailability of PAH in Fresh and Naturally/Artificially Weathered Deepwater Horizon Oil** D. Shea, North Carolina State University / Department of Biology; K. O'Neal, W. Thorsen, P. Lazaro, X. Kong, X. Xia, North Carolina State University. On 20 April 2010, the *Deepwater Horizon* offshore drilling rig in the Gulf of Mexico exploded after a blowout and sank two days later, causing the largest offshore oil spill in U.S. history. One of the ecological and human health issues associated with this spill is the potential for exposure to and accumulation of polycyclic aromatic hydrocarbons (PAH) and other oil components in the food chain and how the use of dispersants may have influenced the bioavailability of PAHs. We will update our preliminary assessment of PAH bioavailability presented at the SETAC Gulf Oil Spill Focused Topic Meeting in April 2011 with final data and conclusions of our field and laboratory experiments. We investigated the bioavailability of PAH in fresh and weathered crude to zooplankton, bivalves, and crustaceans and also tested the ability of passive sampling devices (PSDs) and standard water sampling to predict PAH bioavailability. We found that bioavailability of PAH decreased significantly with the degree of weathering and this effect was most pronounced with lower molecular weight PAH. Use of dispersant increased the bioavailability of fresh crude oil in a manner that appears to be related to the surface area-to-volume ratio of the oil droplets. Various PSD designs were tested and some were subject to a very high bias that was dependent on the presence of oil droplets or films in the water and the ability of the oil to make sustained contact with the PSD sorptive phase. Standard whole and filtered water sampling also was subject to a very high bias and like most PSD designs this bias was highly variable and dependent on the presence oil droplets and films. The potential for this bias was clearly indicated by enrichment of highly water insoluble PAH and hopanes in the sampling media. Our results provide an excellent, though incomplete, basis for determining the bioavailability of PAH as a function of weathering and the appropriateness and potential pitfalls of various sampling technologies to estimate PAH exposure and bioavailability following this oil spill, with clear lessons to be learned for monitoring future oil spills.

**3 A Comprehensive Marine Toxicity Program to Address the Potential Toxicity of Petroleum Hydrocarbons from the DWH Incident in the Gulf of Mexico** G.M. Rand, Florida International University / Ecotoxicology & Risk Assessment; P.R. Gardinali, Florida International University / Department of Chemistry & Biochemistry and SERC; B.A. Stubblefield, Oregon State University, Oregon State University / Environmental and Molecular Toxicology; M. Huddleston, ENTRIX; B.S. Echols, Virginia Tech / Department of Biological Sciences, Florida International University / Southeast Environmental Research Center; M.T. BenKinney, Exponent; O.X.

Pelz, BP / Gulf Coast Restoration Organization, BP Gulf Coast Restoration Organization / GCRO, BP / GCRO. The recent crude oil released into the Gulf of Mexico (or Deepwater Horizon Oil incident) has raised important questions relating to complex mixtures, like petroleum hydrocarbons, the exposure scenarios that are produced in water after their release and how they are assessed to determine their potential toxicity. The existing literature is limited and not consistent with respect to petroleum hydrocarbon toxicity methodology, making it difficult to compare results from different investigators. This presentation provides an overview of a comprehensive marine toxicology program designed to provide a robust empirical toxicity database to: 1) apply directly to the assessment of the potential acute and chronic effects to Gulf of Mexico biota as a result of exposures to different oil types (crude, weathered, dispersed, etc.); 2) validate existing toxicity exposure media, general methods and water systems to provide replicable data; 3) assess the potential effects of oil droplets; and the 4) potential occurrence of UV-enhanced toxicity. A multi-species trophic level testing approach is used with ecologically and economically important native test organisms as well as standard test ---organisms. The presentation will emphasize the importance of methods validation to ensure the reproducibility of results and the comparability of results from different tests. Therefore, data sets will be presented on exposure methods [types of exposures (static vs flow-through systems) and WAF preparations (oil: water ratios, high vs low energy mixing, natural vs synthetic saltwater)], and test conditions (sealed vs open test chambers).

**4 Acute toxicity and cardiac impairment of Deepwater Horizon oil on two pelagic fish species native to the Gulf of Mexico** A. Esbaugh, University of Miami, RSMAS / Rosenstiel School of Marine and Atmospheric Science, University of Texas – Austin, Marine Science Institute; E. Mager, J. Stieglitz, R. Hoenig, University of Miami – RSMAS; J. Incardona, N. Scholtz, Northwest Fisheries Science Center; D. Benetti, M. Grosell, University of Miami – RSMAS. Brood stock for two common Gulf of Mexico pelagic species, Mahi (*Coryphaena hippurus*) and Cobia (*Rachycentron canadum*), were collected off the Atlantic coast of Florida and housed at the University of Miami experimental hatchery. Both species performed natural spontaneous spawning events in captivity that yielded high quality fertilized embryos for toxicity testing. Acute toxicity to dissolved PAHs resulting from Deep Water Horizon oil was examined using a 96 h static exposure set-up, with 1 L volumes and 20 organisms per replicate. Mahi tests were performed on a matrix of oil preparations that included three different oil samples (source, artificially weathered source, and naturally weathered slick oil collected from the Gulf of Mexico) and two different preparation methods (high energy and chemically enhanced water accommodated fractions). A dispersant only test was also performed. Cobia tests on high energy preparations (slick and weathered source) were performed for the purposes of cross species comparison. LC50s for cobia and mahi and relative sensitivity will be reported. Additionally, the relative potency of high energy preparations versus chemically enhanced preparations, and of weathered oil versus source oil will be discussed, as will the results of dispersant only tests. Additional tests were performed on Mahi to investigate sub-lethal cardiac impairment (cardiac edema) at 48 h, which is a well characterized impact of PAH exposure. EC50s based on the presence of edema will also be reported and compared to LC50 data generated using the same preparations.

**5 Assessment of the chemistry and acute toxicity of fresh and weathered MC252 oils: The toxic unit approach** J. McGrath, HDR Inc; L. Faksness, B. Hansen, D. Altin, T. Nordtug, SINTEF; O. Pelz, British Petroleum; D. Di Toro, University of Delaware. WAFs prepared from oils are comprised of the water-soluble components of oil and, as such, they are mixtures of different chemicals. The chemicals of concern for toxicity assessment are typically limited to the monocyclic aromatic hydrocarbons (MAHs) and the polycyclic aromatic hydrocarbons (PAHs) which are structurally classified as Type I narcotics. The target lipid model (TLM) of narcotic toxicity demonstrates that the acute toxicities of these chemicals vary and are correlated with  $K_{OW}$  such that LC50 decreases with increasing  $K_{OW}$ . As a result, it is necessary to use the concentrations of individual chemicals, and not just the total hydrocarbon concentration (e.g., total PAH  $\mu\text{g/L}$ ), to estimate hydrocarbon toxicity. To understand the impact of weathering on oil toxicity, the toxicity of a solution at the water solubility of each component needs to be examined. For each chemical, the maximum toxicity or maximum toxic unit (TU) is equal to its water solubility divided by its water effect concentration



(e.g., LC50). Using the relationship of the solubility of oil components to log  $K_{OW}$ , it is demonstrated that chemicals with a lower log  $K_{OW}$  have a greater maximum toxicity than the chemicals with higher log  $K_{OW}$ . Weathering preferentially removes these chemicals with the lower log  $K_{OW}$  (and higher maximum toxicity) as compared to the chemicals with the higher log  $K_{OW}$  (but lower toxicity). As such, weathering decreases the toxicity. The TLM and TU approaches were applied to toxicity and chemistry data from fresh (i.e., neat) and weathered MC252 oils. The weathered oils included artificially and naturally weathered samples. The artificially weathered oil samples were generated by heating fresh oil to 150 to 300°C. The naturally weathered samples were collected in the Gulf after the Deepwater Horizon accident. In the exposures using 100% WAFs, the observed biological responses of *Acartia tonsa* (e.g., mortality) and *Skeletonema costatum* (e.g., reduced growth rate) decrease as the samples increase in weathering, demonstrating that weathering decreases toxicity. The computed TUs also decrease in the 100% WAF for the more weathered samples which is in agreement with the observed biological responses. The use of TUs allows for a correct interpretation of the observed toxicity and the effect of weathering.

**6 Genomic footprint of the Deepwater Horizon oil spill in resident killifish in the laboratory and field** A. Whitehead, University of California Davis; W. Pilcher, Louisiana State University / Department of Biological Sciences; G. Mayer, Texas Tech University / Institute for Environmental and Human Health; B.D. Dubansky, Louisiana State University / Department of Biological Sciences, Louisiana State University / graduate student; F. Galvez, Louisiana State University / Dept. of Biological Sciences. Gulf killifish (*Fundulus grandis*) are the most abundant vertebrate in coastal marsh ecological communities. Large populations of killifish inhabit Gulf-exposed marsh habitats that are at high risk of contamination from oil spilled in the Gulf of Mexico; indeed much habitat was oiled following the Deepwater Horizon oil spill. For these reasons, these killifish are strategic models for assessing contaminating oil impacts on Gulf coast marshes. We conducted a field study spanning the first four months following the oil spill and one year later, integrated with controlled laboratory exposures to experimentally-weathered oil, to characterize oil spill impacts on the coastal marsh by integrating genomic and physiological indicators of biological effects. In field studies genome expression within livers and gills of resident fish was tracked across space (contaminated and reference sites) and time (pre-oil, during oil, and after oil exposure). Genome expression was most distinct at our only field site out of six that was clearly impacted by oil, and at the peak of oil contamination documented by satellite imagery and analytical chemistry, showing a clear genomic footprint of oil exposure. Divergence in genome expression that coincides with contaminating oil is consistent with genome responses that are predictive of exposure to hydrocarbon-like chemicals and suggestive of physiological and reproductive impairment, and coincide with significant impacts on tissue morphology. Transcriptional responses following exposures to experimentally-weathered oil in the laboratory were predictive of the responses observed in the field, and coincided with damage to the DNA molecule. These studies highlight mechanisms of exposure and effects in a relevant resident species.

**7 Impacts of Deepwater Horizon oil on pelagic fish from the Gulf of Mexico – an overview of testing efforts and results** M. Grosell, RSMAS, University of Miami; A. Esbaugh, University of Miami, RSMAS / Rosenstiel School of Marine and Atmospheric Science, University of Texas – Austin, Marine Science Institute / Post-Doctoral Fellow; E. Mager, University of Miami / Rosenstiel School of Marine and Atmospheric Science / Division of Marine Biology & Fisheries; J. Stieglitz, University of Miami; R. Hoenig, D. Benetti, University of Miami / Rosenstiel School of Marine and Atmospheric Sciences; A. Roberts, University of North Texas; J. Incardona, NOAA Fisheries / Env. Conservation Division; N. Scholz, NOAA Fisheries – Northwest Fisheries Science Center. Yellowfin tuna (*Thunnus albacares*), Mahi (*Coryphaena hippurus*) and Cobia (*Rachycentron canadum*), were exposed to oils collected from the Gulf of Mexico following the Deep Water Horizon (DWH) spill in 2010. Brood stock of these three species were captured and maintained at RSMAS, University of Miami (Cobia and Mahi) or at the IATTC Achotines Laboratory, Panama (Yellowfin tuna). All brood-stock spawned spontaneously allowing for exposure of embryos beginning a few hours post fertilization. Standard toxicity tests were performed indoor under controlled light and temperature conditions on all three species. For Mahi, different oils and approaches for preparing Water Accommodated Fractions (WAFs) exhibited different potency with 48-96 hour LC50s in

terms of total polyaromatic hydrocarbons (PAHs). Natural light enhanced DWH oil toxicity to Mahi embryos and larvae suggesting that toxicity data obtained under laboratory conditions may underestimate DWH impacts on early life stages of pelagic Gulf of Mexico fish species. Mortality in young larval Yellowfin tuna and Mahi was preceded by impaired cardiac development and cardiac function, as evident from edema, shortly after hatch and at very low exposure concentrations. In addition to the observed impacts on early life stages, brief (24 hour) exposures of young juvenile Mahi (30-40 days of age) resulted in significantly impaired swimming performance. The effects levels as well as the overall, order of sensitivity to DWH oils among the three test species will be reported.

**8 Acute effects of fresh and weathered MC252 crude oil and dispersants on mollusc and echinoderm larvae** C. Langdon, Oregon State University / Coastal Oregon Marine Experiment Station; S.M. Pargee, Oregon State University; B.A. Stubblefield, Oregon State University, Oregon State University / Environmental and Molecular Toxicology. Following the Deepwater Horizon accident, a series of acute assays were carried out in which developing embryos of bivalve (Pacific oyster, *Crassostrea gigas*; Kumamoto oyster, *Crassostrea sikamea*, Gallo mussel *Mytilus galloprovincialis*) and echinoderm species (sand dollar, *Dendraster excentricus*; purple sea urchin, *Strongylocentrotus purpurata*) were exposed to samples of fresh and field-collected weathered Mississippi Canyon 252 oil. Labs tests used the water-accommodated fractions (WAF) of weathered (CTC) and fresh (Q4000 and MASS) oil samples (prepared at 1:1000 oil to water ratio), chemically-enhanced WAF (CEWAF) where Corexit 9500 was added at a 1:20 dispersant to oil ratio (based on the suppliers recommended application rate), and dispersant alone. Exposure to WAFs of fresh crude oil resulted in EC10 values (based on larval development) of 15 to 59% WAF for fresh oils. Naturally weathered CTC oil was substantially less toxic for the tested species, with EC10 values >25 or >100% for all but one of the seven studies. Under the conditions of the tests, the addition of dispersant increased toxicity in the tests of all three oil. The EC10 values for Corexit 9500 with all species in tests that met USEPA and ASTM criteria ranged from 8 to 13% (expressed as % dilutions of WAF with added dispersant). Oils with dispersant added showed similar responses to those with Corexit 9500 alone; EC10 values ranged from 0.4 to >25% WAF for Q4000/Corexit 9500 mixtures and from 7 to 13% WAF for CTC/Corexit 9500 mixtures. Observed responses were evaluated based on oil component concentrations (polynuclear aromatic hydrocarbons, saturated hydrocarbon compounds, and volatile compounds) as well as dispersant “marker” components, i.e., di-octyl-sulfosuccinate (DOSS) and dipropylene glycol n-butyl ether (DPnB). Hydrocarbon concentrations causing responses in laboratory tests will be compared with concentrations measured in “field-collected” samples at the time of the accident. Examination of the available data suggests that exposures evaluated in the laboratory toxicity tests include the most highly contaminated conditions observed in the Gulf of Mexico during the DWH incident.

**9 Spatio-temporal trends of organophosphate flame retardants in Great Lakes herring gulls, as well as Lake trout and their convergent aquatic food webs** R.J. Letcher, Carleton University / Department of Chemistry, Environment Canada / Science and Technology Branch, Ecotoxicology and Wildlife Health Division, Ecotoxicology and Wildlife Health Division, Science and Technology Branch / Environment Canada; D. Chen, Carleton University / Chemistry, Ecotoxicology and Wildlife Health Division, Wildlife and Landscape Directorate, Science and Technology Branch, Environment Canada, National Wildlife Research Centre, Carleton University, Ottawa, ON, Canada / Department of Chemistry, Carleton University / Department of Chemistry; S. Backus, D. McGoldrick, Water Quality Monitoring and Surveillance Division, Science and Technology Branch / Environment Canada; L.T. Gauthier, Ecotoxicology and Wildlife Health Division, Science and Technology Branch / Environment Canada. From the early 1960s to present day, organophosphate (triesters) flame retardants (OPFRs) have been produced in high volumes and widely used in plastics, foams, textiles and furniture. With the phase-out of flame retardants such as polybrominated diphenyl ethers (PBDEs), the usage of certain OPFRs has increased (e.g., tris(1,3-dichloro-2-propyl) phosphate (TDCPP) and tris(1-chloro-2-propyl) phosphate (TCP)). In the present study, 15 non-halogenated, chlorinated or brominated OPFRs were analyzed for in egg pools (2010) of herring gulls from 5 colonial sites in the Great Lakes (Chantry Is. (Lake Huron), Fighting Is. (Lake Erie), Agawa Rock (Lake Superior), Toronto Harbour (Lake Ontario) and Gull Is. (Lake Michigan)),

and retrospectively over a 20 year period from 1990-2010. Of the 15 OPFRs analyzed for, TCP and tris(2-chloroethyl) phosphate (TCEP) were found in 2010 samples at concentrations ranging from 5.4 to 30.1 and 1.1 to 7.9 ng/g lipid weight (lw), respectively. For tris(2-butoxyethyl) phosphate (TBEP) and triphenyl phosphate (TPP), the concentrations ranged from n.d. to 39 ng/g and 2.1 to 8.2 ng/g lw, respectively. Spatial trends were not obvious regarding the concentrations of these OPFRs among colony sites. For TCP, TCEP, TPP and TBEP, retrospective temporal trends revealed changes to be highly variable. Eggs from the Chantry Is. had no detectable levels prior to 1998. TPP was only quantifiable in eggs from Chantry Is. and Gull Is. taken in 2008 and 2010. TBEP was quantifiable in eggs from almost every year going back to 1990; however, levels appeared to be increasing for Gull Is. and Agawa Rock up to 55 and 30 ng/g lw, respectively, in 2010. TCEP levels also appeared to be increasing for Agawa Rock and Toronto Harbour egg pools with levels of 30 to 40 ng/g lw in 2008-2010. Comparing the sum-concentrations of these four OPFRs, there were general increases for all sites over the 20 year period. Our results demonstrated that several OPFRs bioaccumulate via the diet (aquatic- and/or terrestrial-based) in gulls and are transferred to their eggs, have been in eggs (e.g. TBEP) for at least 20 years, and are present in eggs at sites throughout the Great Lakes basin. We also present data on 2010-collected Lake trout from western Lakes Erie and Ontario, a purely aquatic top predator, and examine the comparative fate and possible biomagnification of e.g., TBEP, in gulls and trout from their convergent prey species including alewife and rainbow smelt.

**10 In ovo effects of TCP and TDCP on lethality, development, thyroid hormone levels and mRNA expression in chicken embryos** A. Farhat, University of Ottawa; D. Crump, S. Chiu, K.L. Williams, R.J. Letcher, L.T. Gautier, S.W. Kennedy, Environment Canada / National Wildlife Research Centre. Tris(1-chloro-2-propyl) phosphate (TCP) and tris(1,3-dichloro-2-propyl) phosphate (TDCP) are high production volume organophosphate flame retardants used primarily in rigid and flexible polyurethane foams. They have been detected in the environment and biota, including wild avian species, but there has been little effort in measuring their toxicological and molecular effects in birds. Egg injection studies were conducted to determine the effects of TCP and TDCP on pipping success, development, thyroid hormone (TH) levels, and hepatic messenger RNA (mRNA) expression levels of genes associated with xenobiotic metabolism and the TH pathway in domestic chicken embryos. Neither compound reduced pipping success; however, TCP exposure significantly delayed pipping time by 13 and 17 hours at the two highest doses (10,000 and 50,000 ng TCP/g egg), respectively, and reduced tarsus length at 50,000 ng/g. TDCP exposure caused a dose-dependent decrease in head plus bill length, and significant decreases in embryo mass and gall bladder size at 50,000 ng/g. Free plasma T4 levels for TCP-exposed embryos did not change; however, a significant decrease in plasma T4 levels was observed in embryos exposed to 10 000 ng TDCP/g. Neither compound affected free plasma T3 levels or total T4 content in thyroid glands. There was a dose-dependent increase in type I deiodinase mRNA expression following TCP exposure, whereas liver fatty-acid binding protein and cytochrome P450 (CYP) 3A37 mRNA levels were induced only at the 50,000 ng/g dose. CYP3A37 and CYP2H1 mRNA levels were induced in liver tissue of embryos exposed to TDCP. THs play a crucial role in initiating hatching of precocial birds; however, the delay in pipping and decreased growth of TCP-exposed embryos was not accompanied by changes in TH levels. The effects of TDCP on growth, gall bladder development and plasma T4 levels highlight a phenotypic response to exposure. Further research on TCP- and TDCP-mediated effects in birds is warranted in order to understand their mechanisms of toxicity and better assess the health effects associated with exposure for the purpose of risk assessment.

**11 Early Embryogenesis is Susceptible to Developmental TDCP Exposure** S.P. McGee, University of South Carolina / Environmental Health Sciences; E.M. Cooper, Duke University / NSOE; H.M. Stapleton, Duke University / Nicholas School of the Environment; D.C. Volz, University of South Carolina / Department of Environmental Health Sciences. Chlorinated phosphate esters (CPEs) are widely used as additive flame retardants for low-density polyurethane foams, and have frequently been detected at elevated levels within environmental media. The objectives of this study were to begin investigating the potential developmental toxicity of four CPEs commonly used in polyurethane foam – tris(1,3-dichloro-2-propyl) phosphate (TDCP), tris(2-chloroethyl) phosphate (TCEP),

tris(1-chloro-2-propyl) phosphate (TCP), and 2,2-bis(chloromethyl) propane-1,3-diyl-tetrakis(2-chloroethyl) bis(phosphate) (V6). Using zebrafish as an animal model, we first demonstrated that (1) static exposure from 5.25 to 96 hpf to TCEP, TCP, or V6 concentrations as high as 50  $\mu$ M resulted in no significant effects on embryonic development or survival; (2) static exposure from 5.25 to 96 hpf to concentrations of 8  $\mu$ M TDCP and above resulted in a significant increase in mortality ( $LC_{50}$  = 8.5  $\mu$ M) compared to vehicle controls; (3) relative to exposures initiated at 5.25 hpf, TDCP exposures initiated at the start of cleavage (0.75 hpf) or restricted to cleavage alone – 0.75 hpf (2-cell) to 2 hpf (64-cell) – resulted in a significant decrease in the concentration required to induce a similar magnitude of toxicity; and (4) embryos surviving static TDCP exposure exhibited a range of abnormal phenotypes that were first visible at initiation of the hatching period (48 hpf). Following characterization of TDCP toxicity, we showed that TDCP uptake and BDCP formation were not associated with enhanced toxicity following exposures that included the cleavage period of development. Moreover, TDCP exposure did not result in obvious impacts on cell cycle, overall embryo size, or cell morphology during cleavage, suggesting that TDCP mediates toxicity at the sub-cellular- or genomic-level during this stage of embryogenesis. Finally, as TDCP-specific genotoxicity assays have largely been negative *in vivo* and zygotic genome remethylation is a key biological event during cleavage, we investigated whether TDCP altered the status of genomic DNA (gDNA) methylation, and showed that normal gDNA methylation at the end of cleavage (2 hpf) was absent in TDCP-treated embryos. Overall, our findings suggest that the cleavage period is susceptible to TDCP-induced delays in remethylation of the zygotic genome, a mechanism that may be associated with enhanced developmental toxicity following initiation of TDCP exposure at the start of cleavage.

**12 The Flame Retardant  $\beta$ -1,2-Dibromo-4-(1,2-dibromoethyl)cyclohexane ( $\beta$ -TBECH): Fate, Fertility and Reproduction in American Kestrels (*Falco sparverius*)** K. Fernie, Environment Canada / Ecotoxicology and Wildlife Health, Wildlife and Landscape Science Directorate, Science and Technology Branch; S.C. Martenson, McGill University / Avian Science and Conservation Centre; L. Graham, University of Guelph / Department of Animal and Poultry Science; S. Kimmons, McGill University; G. Tomy, Department of Fisheries & Oceans Canada; V.P. Palace, Department of Fisheries & Oceans Canada, Stantec Consultants; I. Ritchie, McGill University; L. Gauthier, Environment Canada; R.J. Letcher, Carleton University / Department of Chemistry, Environment Canada / Science and Technology Branch, Ecotoxicology and Wildlife Health Division. Breeding pairs of captive American kestrels (*Falco sparverius*) were exposed via diet during reproduction to an environmentally relevant concentration of  $\beta$ -1,2-dibromo-4-(1,2-dibromoethyl)cyclohexane ( $\beta$ -TBECH). The  $\beta$ -TBECH isomer was injected into the food source at a daily dosing concentration of 0.239 ng/g kestrel/day (22 pairs); control birds were exposed via diet to the safflower oil vehicle only (24 pairs). The males from eight pairs in each group were exposed for four weeks and sacrificed for testicular analysis; the remaining pairs completed their breeding cycle, with exposure ceasing at the end of incubation (82 days).  $\alpha$ - and  $\beta$ -TBECH appeared to be rapidly metabolized and/or eliminated from fat, liver and plasma samples; both isomers and potential hydroxylated metabolites of  $\beta$ -TBECH (plasma) were undetected. Notwithstanding, compared to controls, pairs exposed to  $\beta$ -TBECH laid fewer eggs ( $p = 0.019$ ) that were lighter (successful eggs:  $p = 0.009$ ). Exposed pairs also demonstrated poorer egg fertility ( $p = 0.035$ ) although testis mass and the number of seminiferous tubules containing final spermatids were similar among males. Reductions in egg production and fertility resulted in decreased hatching success ( $p = 0.023$ ). The  $\beta$ -TBECH-exposed pairs also produced fewer males overall ( $p = 0.009$ ), which may have been related to the increased estradiols maternally deposited in the first egg ( $p = 0.039$ ). These findings demonstrate that  $\beta$ -TBECH may be detrimental for breeding in wild birds receiving similar exposure levels.

**13 In Vitro Screening, and use of an Animal Model, the Zebra Finch, to Identify Possible Endocrine Disrupting Effects of TBECH** H. Currier, Simon Fraser University / Biological Sciences; L.I. Wong, Simon Fraser University / Department of Biological Sciences; J. Elliott, Environment Canada / Pacific Wildlife Research Centre, Science and Technology Branch, Environment Canada / Wildlife Toxicology, Environment Canada / Canadian Wildlife Service; T. Beischlag, Simon Fraser University / Health Sciences; T. Williams, Simon Fraser / Biology. Many brominated flame retardants (BFRs) can be classified as endocrine disrupting compounds.



Various toxicological effects in both humans and animals have been reported. TBECH (1,2- dibromo-4-(1, 2-dibromoethyl) cyclohexane), has recently been detected in the environment and biota, but very little is known of its environmental impacts. To identify whether or not TBECH has any endocrine disrupting capabilities, it was run through an *in vitro* screening process that included real-time PCR techniques in two different cell lines (human ECC-1 endometrial carcinoma, and human LNCaP prostate cancer cells). We were interested in whether or not TBECH affected the expression of human hormone receptor-regulated target genes. Cells were treated with environmentally comparable levels of TBECH alone and in combination with the respective steroid hormones to determine if the compound acts as an agonist, antagonist, or amplifies the effects of the hormones. We investigated the effects of the TBECH diastereomers ( $\alpha$ ,  $\beta$ ,  $\gamma$  and  $\delta$ ) on mRNA and protein accumulation of the prostate-specific antigen (PSA) and prostate-specific membrane antigen (PSMA) and AR protein levels in the human prostate cancer cell line, LNCaP. TBECH  $\gamma/\delta$  was shown to relieve R-1881-induced repression of PSMA expression. However, TBECH  $\alpha/\beta$  alone significantly increased mRNA accumulation of PSMA while TBECH  $\gamma/\delta$  in combination with R1881 further repressed PSMA mRNA accumulation. At the protein level, all four TBECH diastereomers at both 1  $\mu$ M and 10  $\mu$ M relieved repression of PSMA expression induced by R1881, which was inconsistent with our mRNA accumulation results. Furthermore, we found that TBECH  $\gamma/\delta$  induces protein accumulation of AR similar to R1881. To look at how these results might affect growth and development in an animal model, TBECH was then tested in zebra finches. Finch embryos were exposed to TBECH via *in ovo* dosing procedures, and their growth and development was measured to fledging. No significant effects on growth or survival of offspring were observed, however analysis of tissue samples at various growth stages suggests that TBECH may be metabolized too quickly to cause long-term effects. Collectively, these results demonstrate that these chemicals may not have the same properties as classic agonists of the AR but may affect AR levels by up-regulation.

**14 PBDE Metabolites Disrupt the Thyroid System and Impair Development in Embryonic Zebrafish (*Danio rerio*)** L.J. Macaulay, L.V. Dishaw, W. Dong, H.M. Stapleton, Duke University / Nicholas School of the Environment. Polybrominated diphenyl ether (PBDE) flame retardants are biotransformed by mammals into hydroxylated PBDEs (OH-BDEs). These metabolites are known to have thyroid disrupting effects, such as competitive binding to thyroid transporter proteins and nuclear receptors, likely due to their strong structural resemblance to endogenous thyroid hormones. Previous studies have demonstrated that OH-BDEs have significant acute toxicity in zebrafish embryos; however the modes of action are unclear. In addition, our laboratory has demonstrated that OH-BDEs inhibit thyroid deiodinase and sulfotransferase activity. To follow-up on these studies, we examined the effects of several OH-BDE congeners in embryonic zebrafish. Zebrafish embryos were exposed to either aqueous 6-OH-BDE-47, 5-OH-BDE-47, 3-OH-BDE-47, or BDE-47 from 4 hours post fertilization (hpf) to 5 days post fertilization (dpf) at concentrations ranging from 1 nM-10  $\mu$ M. Embryos or larvae were evaluated daily for lethality, abnormality, and hatching success. Whole mount *in situ* hybridization was performed on embryos exposed to 100 nM 6-OH-BDE-47 to examine mRNA expression of Deiodinase (DI) I and II. Exposure to  $\geq 1 \mu$ M 6-OH-BDE-47 resulted in complete mortality after 24 hpf. Delayed hatching and reduction in pigmentation were observed in fish at doses tested in the nanomolar range (100 nM-250 nM) with severe developmental delays at concentrations  $\geq 100$  nM. The  $LC_{50}$  value was calculated to be 143 nM. The most common phenotypes observed in abnormal fish were a reduction or delay in development of melanophores associated with pigmentation, curvature of the spine, tail deformities, and edema of the pericardial and abdominal regions. A significant increase in type 1 DI expression was seen in midbrain of embryos that were treated with 6-OH-BDE-47 relative to control fish at 24hpf, and while not significant, there was a suggestion of an increase in type 2 DI ( $p=0.07$ ). There was also a significant increase of type 1 DI expression in embryos exposed to 100 nM BDE-47. These results indicate that exposure to relatively low levels of OH-BDEs can alter zebrafish embryonic development. The mechanism driving the observed developmental effects may be related to disruption of the thyroid system, specifically deiodinase enzymes. Ongoing studies will measure the accumulation of these chemicals and thyroid hormone levels in embryos using LC-MS-MS and gene expression in tissues.

**15 Levels of Non-Polybrominated Diphenyl Ether Halogenated Flame Retardants in Residential House Dust Samples and Firehouse Dust Samples in California** F.R. Brown, Department of Toxic Substances Control / Environmental Chemistry Laboratory; T. Whitehead, University of California Berkeley / School of Public Health; J. Park, Department of Toxic Substances Control / Environmental Chemistry Laboratory; C. Metayer, P.A. Buffler, University of California Berkeley / School of Public Health; M. Petreas, Department of Toxic Substances Control / Environmental Chemistry Laboratory. Because of concerns over their toxicity, PBDEs are being phased out of production, and other halogenated flame retardants (HFRs) are being substituted. However, there are data to suggest that these HFRs are also toxic, and like PBDEs, are found worldwide. This raises questions about the use of HFRs and the potential for humans to be exposed to them. One potential exposure pathway for many environmental contaminants is through dermal contact with or incidental ingestion of house dust. Our laboratory is measuring the levels of 13 HFRs in dust from both residences and fire house living quarters in California. Methods and Materials Dust samples were collected from the vacuum cleaner bags of homes participating in the Northern California Childhood Leukemia (NCCL) study, and a study of California firehouses. NCCL study samples were collected from 2001 – 2007, and again in 2010. Firehouse dust samples were collected in 2010 and again in 2011. Samples from both studies were subjected to extensive cleanup and analyzed using high resolution gas chromatography/mass spectrometry for PAHs, PCBs, and PBDEs, as well as HFRs. Thirteen HFRs were measured: allyl 2,4,6-tribromophenyl ether (ATE); a- and b-tetrabromoethylcyclohexane (a-,b-TBECHs); pentabromotoluene (PBT); pentabromoethylbenzene (PBEb); hexabromobenzene (HBB); 1,2-bis(2,4,6-tribromophenoxy)ethane (BTBPE); 2,3-dibromopropyl 2,4,6-tribromophenyl ether (DPTE); 2-bromoallyl 2,4,6-tribromophenyl ether (BATE); bis(2-ethylhexyl)tetrabromophthalate (TBPH); 2-ethylhexyl-2,3,4,5-tetrabromobenzoate (TBB); and decabromodiphenylethane (DBDPE); and Tris(1,3-dichloro-2-propyl) phosphate (TDCPP). Results and Discussion Unlike what we observed for PBDEs, these preliminary results (9 of 59 NCCL samples, 8 of 23 Firehouse samples) for HFRs show roughly comparable levels in both house and firehouse dust. All analytes, except for ATE, were detected and TBB, BTBPE, TBPH, and DBDPE were the most abundant analytes. Similar to PBDEs, the levels of TBB, BTBPE, TBPH, and DBDPE in CA house dust are higher than levels found in house dust in the Boston, MA area. The views expressed herein are those of the authors and do not necessarily reflect those of the California Department of Toxic Substances Control.

**16 Photolytic and microbial debromination of BDEs in San Francisco Bay** L.A. Rodenburg, Rutgers, the State University of New Jersey / Department of Environmental Sciences, Rutgers University / Department of Environmental Sciences; B.K. Greenfield, University of California, Berkeley, University of California, Berkeley / Berkeley Center for Green Chemistry; S. Klosterhaus, D. Yee, San Francisco Estuary Institute. Brominated diphenyl ethers (BDEs) are a class of flame retardants that have been classified as persistent organic pollutants under the Stockholm Convention and are targeted for phase-out. Despite their classification as persistent, BDEs undergo debromination in the environment, via both bacterial and photolytic pathways. We examined concentrations of 24 BDE congeners in 233 sediment samples from San Francisco Bay using a factor analysis technique called Positive Matrix Factorization (PMF). PMF analysis revealed five factors, two of which were dominated by congeners with two or three bromines, indicating that they appear to be related to debromination processes. One of the factors was dominated by BDE 15 (4,4'-dibromo diphenyl ether), which is a major product of the photolytic debromination of heavier congeners, and therefore appears to indicate photolysis. The other factor contained high proportions of BDE 7 (2,4-dibromo diphenyl ether) and BDE 17 (2,2',4-tribromo diphenyl ether). Because BDE 7 and BDE 17 have been observed as products of microbial debromination of heavier congeners, this factor appears to represent anaerobic microbial dehalogenation. Together, these two debromination factors represent about 8% of the mass in the data matrix, suggesting the BDEs undergo measurable degradation in San Francisco Bay sediment.

**17 An Integrated Approach Toward Understanding the Environmental Fate, Transport, Toxicity and Occupational Health Hazards of Nanomaterials** V. Grassian, University of Iowa / Department of Chemistry. Nanoparticles, the primary building blocks of many nanomaterials, may

become suspended in air or get into water systems, e.g. drinking water systems, ground water systems, estuaries and lakes etc. Therefore, manufactured nanoparticles can become a component of the air we breathe or the water we drink. One important issue in understanding the environmental fate, transport, toxicity and occupational health hazards of nanoparticles is in characterizing the nature and state of nanoparticles in air, water or in vivo. For the nanoparticles of interest in these studies, metals and metal oxides, it can be asked: (i) will metal oxide and metal nanoparticles be present in air or water as isolated particles or in the form of aggregates? (ii) will metal oxide and metal nanoparticles dissolve in aqueous solution or in vivo? and (iii) under what conditions will metal oxide and metal nanoparticles aggregate or dissolve? As the size regime will be very different depending on the state of the nanoparticles, as dissolved ions, isolated nanoparticles or nanoparticle aggregates, these questions are important to address as it impacts the size regime that needs to be considered or modeled in for example environmental transport or lung deposition models. Furthermore, the effect on biological systems including nanoparticle-biological interactions and toxicity will depend on the state of nanoparticles. In the studies discussed here, macroscopic and molecular-based probes that includes quantitative solution phase adsorption measurements, molecular based probes, light scattering and zeta-potential measurements to investigate the behavior of nanoparticles in aqueous suspensions. We have focused on several different metal and metal oxide nanoparticles including Fe, Ag, Zn, Cu, Ce and Ti. Some of our newest results which focus on aggregation and dissolution, including detailed size-dependent studies, in the presence and absence of organic acids will be discussed. This research is beneficial as it significantly contributes to the growing database as to the potential environmental and health implications of nanoscience and nanotechnology and how nanomaterials will behave in the environment and impact human health.

#### 18 Development of an In Vitro Test and a Prototype Model to Predict Cellular Penetration of Nanoparticles

Y. Chen, Georgia Institute of Technology; D. Capco, Arizona State University; Z. Chen, University of Puerto Rico. Concerns regarding the safety and health effects of engineered nanomaterials arise alongside the booming nanotechnology. This year, we investigated the toxicity mechanisms of selected nanoparticles (NPs) on a variety of organisms. 1) We examined the toxicity of metal oxide NPs to *E. coli* cells; the results showed that the cytotoxicity was quantitatively correlated with the reactive oxygen species (ROS) generation by NPs. The ROS generation mechanism was further elucidated by comparing the electronic structures of the metal oxides with the redox potentials of various ROS generation. 2) We tested the NP toxicity on *Paramecium* and found that surface interactions between NPs and cell surface largely affected the toxicity of metal oxide NPs to *Paramecium*. 3) The toxic effects of different sized hematite NPs on intestinal epithelia were examined and the results indicated that the epithelial integrity was affected by disruption of epithelial structures (e.g. apical microvilli) and cell-cell junctions. Epithelial integrity was also affected at the genetic level as shown by differential expression of genes related to cell junction maintenance, which was assessed by microarray analysis. 4) We explored the potential genotoxicity of NPs by investigating the interactions of NPs (e.g. quantum dots, hematite and silver NPs) with single DNA molecules using scanning probe techniques. DNA conformation was altered after the exposure to NPs with the formation of DNA condensates and DNA loops. The interaction energy between NPs and DNA molecules was computed, using the surface element integration (SEI) approach, to predict the affinity of NPs to DNA. The computational predictions agreed well with the binding phenomena observed by atomic force microscopy (AFM). This work should provide unique insights into the toxic effects of engineered nanomaterials at the cellular level and genetic level.

#### 19 Differential Impacts of Hydroxyl Radical and Singlet Oxygen on the Surface Chemistry and Colloidal Stability of Carboxylated Carbon Nanotubes

X. Qu, Q. Li, Rice University. Carbon nanotubes (CNTs) generate reactive oxygen species (ROS) under solar irradiation, which was hypothesized to cause CNT photochemical transformation in aquatic systems. The resulting changes in surface chemistry are expected to greatly affect their environmental fate and transport. This study investigates the impacts of two major ROS generated by CNTs, hydroxyl radical and singlet oxygen, on the surface chemistry of CNTs. Carboxylated multi-walled carbon nanotubes were exposed to hydroxyl radical and singlet oxygen respectively for varying periods of time. The resulting CNT samples were characterized using X-ray photoelectron spectroscopy in conjunction with

chemical derivatization to determine the evolution of major surface oxygen-containing functional groups. Hydroxyl radical and singlet oxygen were found to have differential impacts on CNT surface chemistry. Hydroxyl radical preferentially reacts with the oxygen-containing functional groups, mainly carboxyl groups, resulting in reduced surface oxygen concentration. On the contrary, singlet oxygen mainly reacts with the graphitic carbon, adding oxygen on the CNT surface. Raman spectrum analyses suggest both ROS create more defects on the CNT surface. The reactions of CNTs with both ROS significantly changed the colloidal stability of CNTs in water. Hydroxyl radical reduced the stability of carboxylated CNTs, while singlet oxygen enhanced its stability as measured by sedimentation experiments. This is consistent with previous findings that CNTs with higher surface oxygen content are more negatively charged and therefore more stable in the aqueous phase. These results suggest that solar irradiation induced ROS, either naturally occurring (e.g., formed by NOM) or self-generated by CNTs, play an important role in the environmental fate and transport of CNTs. These reactions may lead to photochemical degradation of CNTs.

#### 20 Effects of Surface Oxides on the Behavior of Carbon Nanotubes and their influence on the Mobility of Contaminants in Aquatic Environments

J. Yang, Johns Hopkins University / Department of Geography and Environmental Engineering; B.A. Smith, J.L. Bitter, D.H. Fairbrother, Johns Hopkins University / Department of Chemistry; W.P. Ball, Johns Hopkins University / Department of Geography and Environmental Engineering. Oxidized multi-walled carbon nanotubes (O-MWCNTs) are commercially available materials that will be increasingly found in natural waters, where their fate is important to understand in contexts of subsurface transport and engineered filtration. In this work our interest is in the transport and retention of both O-MWCNTs and co-occurring contaminants that adsorb to the O-MWCNTs. Our project therefore includes studies of both transport and sorption. In regard to transport through porous media, deposition processes are well understood for spherical particles moving in clean beds of spherical "collectors"; however, the associated theory has not yet been well tested for extremely high-aspect nano-materials such as O-MWCNTs. We have studied the transport and deposition of O-MWCNTs in well-defined silica-based media as a function of MWCNT surface oxidation and with varying aquatic chemistry. Temporal breakthrough curves and spatial retention profiles were obtained and evaluated using numerical models of particle transport. Fitting such models to both spatial and temporal data allows us to explore the relevance of current theory to MWCNTs and to also explore alternative modeling approaches. In regard to adsorption, our study has included batch isotherm work using both naphthalene and  $\text{Zn}^{2+}$  with O-MWCNTs as sorbents, toward understanding how such sorption can lead to facilitated transport or retention of the dissolved species during flow through porous media. For both contaminants, non-linear adsorption isotherms were fit to the data and effects of solution chemistry were observed to follow predictable trends. Complications were discovered, however, with regard to the impact of solid-to-liquid ratio and aggregation state. These issues are still under investigation. With independent understanding of deposition, transport, and co-contaminant adsorption (to both O-MWCNTs and media solids), we are able to parameterize a transport model that includes synergistic or antagonistic effects of O-MWCNTs on chemical pollutant transport. In addition, the presence of some co-solutes such as natural organic matter (NOM) can have dramatic effects on O-MWCNT properties, and related studies are underway. Although we expect the NOM-related phenomena will be more difficult to numerically model, the experimental results should provide valuable insight into combined transport under complex realistic conditions.

#### 21 Elucidating the mechanisms by which nanoparticles interact with membrane receptors and enter mammalian cells

R.R. Garner, Clemson University / Institute of Environmental Toxicology (CU-ENTOX), Clemson University / Institute of Environmental Toxicology; W.S. Baldwin, Clemson University / Institute of Environmental Toxicology; A.S. Mount, Clemson University / Department of Biological Sciences; S.J. Klaine, Clemson University / Institute of Environmental Toxicology (CU-ENTOX), Clemson University / Institute of Environmental Toxicology (ENTOX), Clemson University / Institute of Environmental Toxicology (ENTOX). There are currently over 1000 consumer products on the market that contain or utilize nanomaterials, and while these materials have been shown to cross cellular membranes, little research has examined the processes by which this occurs. Previously, we reported on the effects of particle

size, shape, and surface chemistry on cellular uptake of gold nanoparticles. The goal of this project was to characterize the mechanism of cell uptake for particle-serum protein complexes. Particles were characterized by TEM, DLS, UV-vis, and zeta potential in both their stock solutions as well as the exposure media. A549 carcinomic human alveolar cells were plated in 12-well plates at 100,000 cells per well and exposed to 1 mg/L gold for 0.5, 1, 1.5, and 2 hours. Fetal bovine serum (FBS) and Bovine Serum Albumin (BSA) were used as protein sources. Treatments varied by the amount and kind of protein source. Bioaccumulation of nanoparticles at each time point was quantified by inductively coupled plasma-mass spectrometry (ICP-MS), and movement was visualized through environmental scanning electron microscopy. Particle uptake was inversely related to FBS concentration ( $R^2=0.87$ ,  $p < 0.001$ ). Conversely, particle uptake increased as a function of BSA concentration ( $R^2=0.89$ ,  $p < 0.001$ ). This data suggests that albumin, one of a vast array of components in FBS, is associated with the transport of particles across membranes, and that other components of FBS may inhibit uptake due to their competition for particle complexation and inability to interact with membrane bound receptors. Particle uptake decreased when exposed in calcium free media, supporting the role of calcium in particle uptake. Cells were further exposed in ATP and potassium depleted media to determine if receptor mediated endocytosis was involved particle uptake. Finally, membrane receptors TGF- $\beta$ , megalin, and cubilin, which are known to interact with albumin, were inhibited and bioaccumulation was examined to further explore the mechanisms of nanoparticle uptake in cells. This research provides fundamental information on the interactions of nanoparticles with cells. Further, the utility of this high-throughput bioassay to screen large number of nanoparticles will facilitate future risk assessment endeavors.

**22 Environmental transformation and biological fate of fresh and aged cerium oxide nanoparticles** M. Morishita, The University of Michigan / Environmental Health Sciences; O. Joliet, The University of Michigan / School of Public Health, University of Michigan / Environmental Health Sciences, School of Public Health; D. Li, The University of Michigan / Environmental Health Sciences; M. Wooldridge, E. Eagle, E. Bumbalough, The University of Michigan / Department of Mechanical Engineering; J. Wagner, J. Harkema, Michigan State University / Department of Pathobiology and Diagnostic Investigation; C. Emond, University of Montreal. Cerium oxide ( $\text{CeO}_2$ ) has been used in petroleum refining and automotive catalytic converters, and  $\text{CeO}_2$  nanoparticles (NPs) are increasingly being employed as an additive to promote diesel fuel combustion in other countries. However, little is known regarding the mechanisms of transformation of  $\text{CeO}_2$  NPs in the atmosphere or their biological fate. The overall objective of our project is to improve our understanding of environmental exposure-dose pathways of  $\text{CeO}_2$  NPs. Our Specific Aims are: (1) characterize environmental transformation and physicochemical properties of aged  $\text{CeO}_2$  NPs using their interactions with UV radiation and ambient air co-pollutants, and compare them to freshly-combusted  $\text{CeO}_2$  NPs; (2) determine the biological fate of freshly-combusted and aged  $\text{CeO}_2$  NPs, comparing the concentrations in blood and target organs resulting from animal inhalation and intratracheal exposures; and (3) develop and evaluate a Physiologically Based Toxicokinetic (PBTK) model of  $\text{CeO}_2$  NPs to identify the main factors affecting translocation and distribution of  $\text{CeO}_2$  NPs in the body. We are currently conducting detailed physicochemical characterization of freshly-combusted  $\text{CeO}_2$  NPs and will next measure the degree of photochemical transformation during interactions with UV radiation and air co-pollutants. We will then conduct animal inhalation and intratracheal instillation exposure studies, and measure  $\text{CeO}_2$  NP concentrations in blood and multiple organs. Physicochemical characterization and the progress on animal exposure studies will be presented. The project will provide new insights into physicochemical transformation of  $\text{CeO}_2$  NPs in the atmosphere, biological fate and mass balance of  $\text{CeO}_2$  NP distribution via inhalation exposure routes, and the first PBTK model of environmentally relevant  $\text{CeO}_2$  NP inhalation exposure.

**23 Flexible Poly(amic) Acid Membrane Captures, Isolates & Simultaneously Detects Engineered Nanoparticles** W. Sadik, State University of New York at Binghamton / Chemistry Department, State Univ. of New York at Binghamton / Professor of Chemistry; N. Du, State University of New York-Binghamton / Chemistry; V. Okello, State University of New York-Binghamton / Department of Chemistry. The goal of this project is to develop nanocavity sensor (category II) arrays for the isolation, detection

and quantitation of engineered nanoparticles (ENPs) in complex environmental matrices. There is urgent demand for rapid screening methods to isolate, detect, and monitor engineered nanomaterials in the environment. Conventional methods for characterizing nanomaterials such as transmission electron microscopy, scanning electron microscopy and atomic force microscopy tend to be bulky and inadequate for field & rapid screening of free nanomaterials<sup>1</sup>. At SUNY-Binghamton, we have developed a new class of nanostructured poly(amic acid) -PAA-membranes that are conductive and electroactive by preventing its imidization to polyimide, while retaining its carboxylic acid and amine functionalities<sup>2-4</sup>. We have studied the effect of composition and microstructure on the optical and electrochemical properties of PAA hybrid composites. The uniqueness of PAA lies in its excellent physical and chemical properties: transparency, flexibility, electrical conductivity, and accessibility to forming large-area device. During the reporting period, our Group discovered that this new class of flexible, stand-alone membranes could be successfully used as both sensors and nanofilters. A new nanofilters device based on PAA membranes is hereby introduced. The nanofilters were derived from phase-inverted, copolymers of PAA and other polymers, with the surface and pore sizes systematically controlled by varying the conditions of the synthesis. This presentation will focus on the use of PAA membranes for simultaneous removal and electrochemical detection of silver nanoparticles, quantum dots and titanium dioxide nanocrystal from food supplements and environmental samples.

**24 Qualification and quantification of engineered nanoparticles in municipal wastewater systems** S. Choi, University of Delaware / Civil and Environmental Engineering; J. Eichhorn, M.V. Johnston, University of Delaware / Chemistry and Biochemistry; C. Huang, University of Delaware / Civil and Environmental Engineering. In spite of the mass effort focused on the development of engineered nanoparticles (ENPs), the amount of discharge into and the effect of ENPs on the environment are still yet to be known. This mainly can be attributed to the technical difficulties involved in the isolation and detection of ENPs in water and wastewater. This lack of technique for the study of free ENPs in wastewater treatment plants has prompted us to carry out the following investigation. The main focus of the research was on developing an appropriate methodology for detecting the distribution of engineered nano particles in municipal wastewater treatment systems. Sludge was sampled from various points in several regional municipal wastewater treatment facilities including the city of Wilmington, DE, Philadelphia, PA, Baltimore, MD, and Washington DC. The sludge samples, upon collection, were first cleaned using advanced oxidation process, by autoclaving in the presence of hydrogen peroxide, to remove the organic and biological constituents. The organic-free sludge samples were further cleaned using dialysis to remove salts. The remaining materials, mostly inorganic by nature, are further characterized for physical and chemical properties as to identify and quantifying the ENPs. Scanning electron microscopy (SEM) and energy dispersive spectroscopy (EDS) were used to gain information on the type, size, and chemical composition of the ENPs. Inductively coupled plasma optical emission and mass spectroscopy (ICP-OES and ICP-MS) technique were used to identify and quantified the ENPs. Additionally, scanning mobility particle sizer (SMPS) coupled with LiquiScan NP nebulizer and condensation particle counter (CPC) was used to classify the particle size of ENPs. Results showed the presence of titanium, zinc, iron, and aluminum nanoparticles throughout the entire wastewater treatment systems, namely, secondary influents, aeration tank, secondary effluents and sludge. Concentration of metallic ENPs up to a level of 600 mg/kg -dried sludge was observed. Results also showed that the ENPs in the sludge had an average diameter of 10-20 nm and the particle size decreased with the degree of dilution with water indicating the aggregation nature of the ENPs in the sludge matrix. The number concentration also increased as the degree of dilution increased, which confirmed the destabilization of aggregates upon dilution.

**25 Exposure pathways for personal care products** D. Mackay, Trent University, Trent University / Environmental Studies; L. Hughes, Trent University / Environmental Studies. Exposure pathways to Personal Care Products (PCPs) are fundamentally different in nature from those of most conventional widely dispersed organic substances such as organo-chlorines and PAHs. The PCP ingredients may be applied directly to humans both outdoors and in the limited ventilation conditions indoors resulting in a significant pulse of exposure, dermally and by inhalation. Ingredients used domestically may be discharged to waste water treatment systems resulting



in measurable levels in treatment plants, in aquatic receiving ecosystems such as rivers and in sludges applied to soils. It is argued that there is a need to quantify these personal and environmental pathways to provide a picture of the relative magnitudes of the key exposure routes. To accomplish this four sets of data are required: (1) per capita usage information, (2) accurate partitioning and reactivity data, (3) results from well designed monitoring programs and (4) predictions of fate and pathways of exposure from mass balance models. Suitable models are described and the need for complementary uncertainty analyses is emphasized. The aim is to achieve consistency between analytical results and model predictions that are a sound basis for risk assessment, improved ingredient selection and possible regulation. This suggested assessment strategy is illustrated by a case study of the cyclic methyl siloxane, D5. This PCP has been the subject of recent risk assessments by Health Canada and Environment Canada and an independent Board of Review. The findings illustrate the need for credible and quantitative determination of exposure pathways based on the four components outlined above. Only then can estimates of toxicity or adverse effect levels be exploited to provide a meaningful assessment of risk.

## **26 ScenAT: a geo-referenced exposure model for assessing the aquatic risk of personal care ingredients in Asia**

R. Vamshi, C.M. Holmes, Waterborne Environmental, Inc.; J.E. Hodges, Unilever / Safety and Environmental Assurance Centre; O. Price, Unilever; M. Rowson, T. Miah, Unilever / Data Sciences. Economies across Asia are growing rapidly as has the consumer demand for a number of personal care products. Consequently, the quantity and detection of chemicals used in personal care products is increasing in profile across a large geographic expanse, such as Asia. However, to date few tools have been developed to predict chemical exposure in water bodies across Asia. Data required to predict relative environmental concentrations (PEC's) of ingredients used in personal care products include emission estimates, properties of the ingredient, socioeconomic and environment data (e.g. receiving water bodies, sewage treatment plant (STP) infrastructure). The majority of tools that have been developed over the past 30 years to assess exposure of chemicals used in personal care products have been developed in Europe and North America and little information is available to parameterise exposure models in Asia for use in environmental risk assessment. Over the past 18 months a novel geo-referenced exposure model has been developed for twelve countries in Asia (the "Asia 12": People's Republic of China, India, Bangladesh, Sri Lanka, Thailand, Laos PDR, Cambodia, Vietnam, Malaysia, Indonesia, Pakistan and the Philippines) to predict the exposure of personal care ingredients in freshwater ecosystems. We explore novel approaches to estimating chemical usage across the diverse continent using information on population, GDP distributions and the population's ability to purchase personal care products. The Koppen-Geiger classification for climate was used to characterise nine climate zones across the Asia 12; we then explore the driving variables in exposure within countries and across these diverse climatic zones. We present our data sources, model development and the utility of the model for predicting relative chemical exposure for a range of ingredients used in personal care products across Asia 12 and its application within a probabilistic risk assessment framework. The results of this exercise can be used to target future monitoring campaigns and could be used to parameterise regulatory models for use in environmental risk assessments for countries in Asia. Please note: This abstract has been submitted for presentation at SETAC-Asia Pacific (2012) by one of the authors listed. But is being submitted here to target and reach a totally different set of audience.

## **27 Evolution of the model SimpleTreat to predict the fate of personal care product ingredients in the sewage treatment plant**

A. Franco, Unilever / SEAC; J. Struijs, RIVM / Laboratory for Ecological Risk Assessment; O. Price, Unilever / SEAC; T. Gouin, Unilever / Safety and Environmental Assurance Centre. Sewage treatment plant (STP) models such as SimpleTreat are used in regulatory risk assessment to predict the discharge of personal care product (PCP) ingredients into the environment. SimpleTreat describes the fate of organic chemicals in a conventional activated sludge STP with primary and secondary sedimentation. Although the model is recommended in the EU regulatory context for personal care product ingredients, biocides and pharmaceuticals, concerns have been raised about a) the applicability domain to chemicals of emerging concern (such as those used in personal care products) and b) the lack of realism of the model default (worst case) parameterisation. Efforts have been made to evolve SimpleTreat and enlarge its applicability domain and to improve its parameterization to

achieve realistic estimation of the concentrations of chemicals in effluent water and sludge. A probabilistic parameterization, based on actual STPs conditions for the EU, was applied to achieve a more realistic representation of the variability of raw sewage characteristics, STP design and operational parameters. The uncertainty of chemical input properties, including degradation rates derived from continuously stirred activated sludge simulation tests (OECD 303A) was incorporated in the simulations. A validation study with ten selected chemicals from PCP ingredients showed good agreement with measured concentrations collected from the literature. The sludge loading rate, the sewage flow, the water temperature and the concentration of suspended solids in the effluent were the most sensitive model parameters and explained, although not completely, the variability in observed removal rates.

## **28 Relationship of Land Uses with Occurrence of Contaminants of Emerging Concern in Streams of Southeastern Minnesota**

D. Fairbairn, E. Spande, University of Minnesota / Water Resources Center; P. Rice, United States Department of Agriculture – Agricultural Research Service; W. Arnold, P. Novak, University of Minnesota / Civil Engineering; W. Koskinen, United States Department of Agriculture – Agricultural Research Service; B. Barber, University of Minnesota / Department of Soil, Water, and Chemistry; D. Swackhamer, University of Minnesota / Environmental Health Sciences. Endocrine disrupting compounds (EDC), pharmaceuticals, and other contaminants of emerging concern (CEC) have been detected in surface waters, including compounds suspected or known to cause adverse human or ecological effects. Goals of the project are to (1) characterize CEC profiles and land uses associated with sub-watersheds in the study area and (2) identify CEC-land use "fingerprints" (unique profiles of chemical markers) to indicate the influence of a given land use on water quality. Our approach includes collecting water (grab and time-composited), passive organic contaminant integrative sampler (POCIS), and sediment samples, ten weeks per year for two years, at four sites near Rochester, MN. Sample extracts (solvent and/or solid phase) are analyzed using liquid chromatography tandem-mass spectrometry for thirty CECs including pesticides, natural and synthetic hormones, household and industrial chemicals, pharmaceuticals and personal care products, veterinary medications, and phytoestrogens. Land use analysis (McGhie Betts, Inc), water chemistry, and flow data accompany the CEC data. CEC concentration profiles are analyzed in light of land use variations amongst subwatersheds, seasonal conditions, precipitation patterns and other hydrologic factors. Sampling began in 2011 and the project will be completed in June 2013. Anticipated results will provide tools to identify sources of surface water contaminants in Minnesota and other locations, providing insight for targeting the most effective mitigation approaches.

## **29 The ecological risk of naturally-occurring and anthropogenic sources of person care product ingredients: fatty alcohols and related surfactants**

S.M. Mudge, Exponent; P.C. DeLeo, American Cleaning Institute / Environmental Safety, American Cleaning Institute. Fatty alcohols are one of the major surfactant classes used in personal care products. These compounds are also synthesised naturally by a wide range of organisms and give rise to a complex mixture of chain lengths and ligands in the environment. The fatty alcohol-based detergents used in personal care products may occur as free alcohols, or as more complicated sulphated, ethoxylated and ethoxy-sulphated compounds which may degrade into free alcohols later found in the environment. They tend to be in the chain length range of C<sub>12</sub> to C<sub>16</sub> and are made from both petrochemical and oleochemical carbon sources. This chain length range overlaps with natural bacterial production as well as algal synthesis through the fatty acid synthase system. In contrast, terrestrial plants manufacture longer chain compounds, typically with chain lengths greater than C<sub>22</sub>. These compounds tend to be in the form of waxes used to prevent desiccation. Free fatty alcohols are relatively insoluble in water and the water solubility is a function of chain length. Compounds with an alkyl chain greater than C<sub>14</sub> are functionally insoluble in water as the Log K<sub>ow</sub> exceeds 6. This leads to strong particle associations in environmental systems with little available for biological uptake. Marketing data on the use of products containing fatty alcohols and measurements made in a wide range of sewerage systems has quantified the inputs and liquid discharges to receiving waters. The chain length profiles and stable isotopes indicate that the compounds in the effluent are not the same as those in the influent having been synthesised during the biological treatment stage. The environmental profiles also indicate a very small contribution from the wastewater

treatment plants (WWTPs) to the local sediments. The bulk of the fatty alcohols that enter the WWTP are removed in the solid phase which may undergo further treatment before being disposed of elsewhere. This may include spreading on agricultural land as a fertiliser. The ecological risk to the rivers from these compounds is very small, and the anthropogenic contribution to that risk is even smaller; the major component (84%) can be attributed to naturally occurring terrestrial plants. The bulk of the shorter chain compounds are bacterial or algal in origin and again are naturally occurring. The small component that may be attributed to discharges from the WWTPs is substantially under the PNEC for the free alcohols based on QSAR approaches.

**30 Impact of Triclosan in the Terrestrial Environment: A Comprehensive Evaluation of Terrestrial Risk** D. Fort, Fort Environmental Laboratories, Fort Environmental Laboratories, Inc.; M. Mathis, Fort Environmental Laboratories; S. Pawlowski, S. Champ, BASF SE. A comprehensive evaluation of the effects of the antimicrobial personal care product triclosan (TCS) in the terrestrial environment was performed. This evaluation evaluated the effects of TCS on acute and chronic toxicity to earthworm (*Eisenia fetida*), and effects on emergence and growth terrestrial plant species. In addition, the effects of TCS on soil microflora respiration and nitrification and the impact on terrestrial arthropods were also considered. The acute and chronic toxicity of TCS to earthworms were tested in an artificial soil in accordance with OECD guidelines 207 and 222. Test concentrations for acute and chronic toxicity studies with earthworms ranged from 64-1,026 and 0.3-100 mg/Kg soil (dw) for acute and chronic studies, respectively. Phytotoxicity studies evaluating survival, emergence, shoot biomass and length, and normalcy of development in 10 species of terrestrial plants were performed in accordance with OECD guideline 208 using 0.2-1,000 mg/Kg (dw) TCS for an exposure period of the median emergence time plus 14 days. Microbial respiration and nitrification were evaluated in soil treated with 0.1-2.0 mg/Kg TCS for 28 days. The effects of TCS on the predatory mite *Hypoaspis aculeifer* exposed to 0.3-320 mg/Kg (dw) TCS for 14 days were evaluated in accordance with OECD guideline 226. NOEC and LOEC values for acute and chronic toxicity to earthworms were 1,026 and >1,026 mg/Kg (dw) [survival], and 100 and >100 mg/Kg (dw) [survival and reproduction], respectively. NOEC and LOEC values in terrestrial plants ranged from 75 and 100 mg/Kg (dw) [biomass in lettuce] to 1,000 and >1,000 mg/Kg (dw) [emergence and phytotoxicity in vetch and phytotoxicity in cucumber]. No Observed Effects Concentrations (NOEC) and Lowest Observed Effects Concentrations (LOEC) for soil respiration and soil nitrification were 2 and >2 mg/Kg (dw), respectively. NOEC and Lowest Observed Effects Concentrations (LOEC) values for predatory mite survival and reproduction were 10 and 32 mg/Kg (dw) and 3.2 and 10 mg/Kg (dw), respectively. Overall, these studies demonstrate that TCS poses minimal risk in the terrestrial environment. These results will be discussed in the context of the terrestrial ecological risk assessment of TCS described by Reiss et al. (2002).

**31 Analysis of Pharmaceuticals and Personal Care Products in Marine Sediment and Biota Using Liquid Chromatography-Tandem Mass Spectrometry** F. Chen, National University of Singapore, National University of Singapore / Department of Civil and Environmental Engineering, National University of Singapore / Civil and Environmental Engineering; S. Bayen, University of Singapore; B.C. Kelly, National University of Singapore / Department of Civil and Environmental Engineering. The discharge of pharmaceuticals and personal care products (PPCPs) into the environment via household, municipal and industrial wastes is a pressing public health issue. Some of these chemicals have been reported as toxic to aquatic organisms in the literature and high environmental concentrations may cause toxic effects. Here we present the development of an analytical method for quantitative determination of key PPCPs, including 35 pharmaceuticals and 7 personal care products, in marine sediments and biota from Singapore's marine environment. Field collected sediments (2 g) and biota tissue (2-3g) samples were extracted and cleaned up by sonication and solid phase extraction (SPE). Identification and quantification of target compounds was performed using liquid chromatography-electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS). Separate analyses were conducted for negative and positive ionization mode. Isotopically labeled ( $^{13}\text{C}$  or deuterated) surrogate standards were used to correct matrix effects. Matrix effects (ionization suppression and enhancement) in sediment and tissue extracts were further assessed for the various analytes by standard addition tests.

Results of analyte recovery tests, procedural blanks and replicate sample analyses demonstrate generally good performance of the methods for the majority of target analytes. Trace residues of several PPCPs, including Caffeine, Carbamazepine, Diclofenac, Diphenhydramine, Estrone (E1), Estradiol (E2), Gemfibrozil, Ibuprofen, Linuron, Naproxen, Simvastatin and Triclosan, were detected in sediments and biota. The significance of the occurrence, levels and patterns of various PPCPs in Singapore's marine environment are discussed.

**32 Solid Waste Deposits as a significant source for contaminants of emerging concern to the environments – a case study from Owerri, Nigeria** A. Arukwe, Norwegian University of Science and Technology (NTNU) / Department of Biology, Norwegian University of Science and Technology (NTNU) / Biology, NTNU / Dept. of Biology; T. Eggen, Bioforsk, Norwegian Institute for Agricultural and Environmental Research; M. Moeder, Helmholtz Centre for Environmental Research UFZ / Department of Analytical Chemistry. There are needs for scientific basis in developing countries, to sensitize communities on the problems arising from improper solid waste deposition and the acute and long-term consequences for areas receiving immobilized pollutants. In Nigeria, as in many other African countries, solid waste disposal by way of crude dumping has been the only management option of such wastes. In the present study, we have highlighted the challenges of solid waste deposit and management in developing countries, focusing on contaminants of emerging concern (including endocrine disrupting compounds: EDCs) and leaching into the environment. We have analyzed sediments and run-off water samples from a solid waste dumping site in Owerri, Nigeria for organic load and compared with representative world cities. Learning from previous incidents, we intend to introduce some perspective for awareness of contaminants of emerging concerns such as those with potential endocrine disrupting activities in wildlife and humans. Qualitative and quantitative data obtained by GC-MS provided an overview on lipophilic and semi-polar substances released from solid waste, accumulated in sediments and transported via leachates. The total ion current (TIC)-chromatograms of sediment extracts clearly point to contamination related to heavy oil. The homologous series of n-alkanes with chain lengths ranging between C16 and C30, as well as detected PAHs such as anthracene, phenanthrene, fluoranthene and pyrene support the assumption that diesel fuel or high boiling fractions of oil are deposited on the site. Targeted quantitative analysis for selected EDCs and pharmaceuticals showed high concentration of substances typically released from technical products such as plastics, textiles, household and consumer products. Phthalates, an integral component of plastic products, was the dominant compound group in all sediment samples and the run-off water sample. Technical nonylphenols (mixture of isomers), metabolites of non-ionic surfactants (nonylphenol-polyethoxylates), UV-filter compound ethyl methoxy cinnamate (EHMC) and bisphenol A (BPA) were particularly determined in the sediment samples at high  $\mu\text{g/kg}$  dry weight concentration. Overall, measuring contaminants in such areas will help in increasing governmental, societal and industrial awareness on the extent and seriousness of the situation both at contaminated sites and surrounding environments.

**33 A real-time antibody-based field assay to predict PAH bioaccumulation in oysters exposed to contaminated sediments** M.A Unger, Virginia Institute of Marine Science / Environmental and Aquatic Animal Health; S.L. Kaattari, Virginia Institute of Marine Science / Environmental and Aquatic Animal Health, Virginia Institute of Marine Science / Professor; W.K. Vogelbein, Virginia Institute of Marine Science / Environmental and Aquatic Animal Health; J. Rieger, The Elizabeth River Project. Lipophilic contaminants in sediments commonly accumulate in shellfish, potentially posing significant human health risk when consumed. Polycyclic aromatic hydrocarbon (PAH) bioaccumulation in shellfish is driven by lipid partitioning but multiple chemical, physical and environmental factors influence bioavailability and tissue concentrations in dynamic natural systems. Because measuring contaminant uptake in biota is time consuming and expensive, multi-phase models have been developed to predict contaminant fate and disposition. However, temporal variability and heterogeneity of natural habitats make it difficult to reliably predict bioaccumulation from measured bulk sediment concentrations and properties. Ultimately, site-specific pore water measurements are vital to accurately predict contaminant bioavailability and to evaluate the effectiveness of sediment remediation efforts. Recent advances in biosensor technology now allows near real-time measurement of contaminants at sub part per billion concentrations. A



quantitative, monoclonal antibody (mAb)-based sensor was used to measure PAH concentrations in sediment-associated water as a predictor of oyster tissue burdens. Oysters, sediment, surface water and sediment pore waters were collected at six sites in the Elizabeth River, Virginia that included a Superfund site and sites undergoing sediment remediation. PAH concentrations in surface water and sediment pore water were measured in the field within minutes of collection by biosensor. Total PAH concentrations ranged from  $< 1 \mu\text{g/L}$  to  $>100 \mu\text{g/L}$  in these aqueous samples. The relationships between measured aqueous PAH concentrations, oyster bioaccumulation, sediment properties and sediment remediation methods will be discussed.

**34 Addressing Risk Assessment Needs for Traditional and Emerging Contaminants Using the Innovative In Situ Sampling/Bioavailability (IS2B) Device** R.U. Halden, Arizona State University / Center for Environmental Security, The Biodesign Institute at Arizona State University; S.D. Supowit, Arizona State University / Department of Civil and Environmental Engineering; I.B. Roll, Arizona State University; V.D. Dang, K.J. Kroll, University of Florida; N.D. Denslow, University of Florida / Department of Physiological Sciences and Center for Environmental and Human Toxicology, University of Florida / Dept. of Physiological Sciences &. To address pressing needs in environmental risk assessment, we introduce the *in situ* sampling/bioavailability determination (IS2B) tool, a novel, patent-pending device designed to allow for simultaneous determination of contaminant levels in bulk water and pore water at hitherto unattainably low method detection limits (MDLs). When deployed, one half of the tubular IS2B device is buried in sediment and the other is exposed to bulk water. Integrated multi-channel pumps simultaneously draw bulk water and sediment pore water into the active sampling device and push it at, respectively, high and low desirable flow rates through an array of filters and adsorption media. Water samples either can be stored in the device or expelled into the bulk water at will. Due to unlimited access to pore and bulk water during deployment, the IS2B tool has the potential to provide ultra-low MDLs for a broad spectrum of contaminants, ranging from fully water-soluble to highly sorptive and hydrophobic. The working hypothesis of our research team is that the IS2B can serve to reliably (i) sample bulk and pore water pollutants for analysis in the sub-ng/L range, (ii) inform on the bioavailability and bioactivity of sediment contaminants, (iii) inform on human exposures and associated health risks from fish consumption, and (iv) track the progress of sediment remediation activities. We provide an overview of the device principles, preliminary performance data, and a set of laboratory and field studies designed to systematically assess the utility of the IS2B tool in contaminated sediments from Lake Apopka, Florida. To assess the breadth of IS2B applicability, the project utilizes mesocosm experiments with two traditional and three emerging contaminants (*p,p'*-DDE, dieldrin vs. fipronil, triclosan, triclocarban) and involves two test organisms: *Lumbricus variegatus* and *Pimephales promelas*. Biological responses to contaminant exposure are measured in fish using DNA microarray analysis to evaluate effects that may not be predicted solely based on body burden of parent compounds. Finally, the project involves the development of mathematical relationships between pollutant concentrations in bulk water, pore water, worms and fish to quantify exposure risks and track the effectiveness of two remediation approaches, granular activated carbon (GAC) amendment and deep tilling of contaminated sediment.

**35 Applications of Sediment Bioavailability Tools to Human Health Risk Assessment** T. Sorell, AMEC / Environment and Infrastructure; M. Murphy, AMEC. There is an expanding universe of tools used in sediment investigation to assess bioavailability to biota. These tools, which include direct measurement of pore water contaminant of concern (COC) concentrations (both dissolved and freely dissolved), measurement of ligands and other chemical parameters such as dissolved organic matter to estimate adsorption, and models to predict food chain transfer based on site-specific physical and chemical conditions, have become routine in predicting ecological risk. For example, it has become widely accepted that pore water concentrations correlate with toxicity to benthic organisms. However, application of these critical observations to human health risk is not routine. Rather, human health assessments tend to rely on default assumptions and food chain transfer factors derived from publicly available databases, empirical observations at other sites, or simplistic models that do not account for site chemistry. Site-specific sediment and pore water chemistry may be useful in evaluating and possibly modifying default values, such as food chain transfer factors, oral absorption efficiency, and dermal absorption factors.

Critical bioavailability-related considerations for assessing human health risk from sediment sites: Relationship between adsorbed and freely soluble COC thermodynamic equilibrium and assimilation at the base of the food chain Biomagnification behavior of the COC Importance of sediment ingestion as an assimilation mechanism in the local food chain Tendency of the COC to be metabolized Sensitive variables in food chain transfer models The routes of human exposures (biota consumption, direct contact via incidental ingestion and dermal contact) Relevance of adsorptive behavior in situ to human absorption via gastrointestinal and dermal exposure routes The evaluations presented here explore the mechanisms by which site information typically collected for ecological risk purposes may be of predictive value in the human health risk assessment process. Applying site bioavailability-related observations into the human health assessment may avoid over-estimation of exposure and provide integrated risk evaluations that support balanced risk management decisions.

**36 Bioavailability-based toxicity endpoints of bifenthrin for *Hyalella azteca* and *Chironomus dilutus*** A.D. Harwood, Southern Illinois University / Fisheries and Illinois Aquaculture Center and Department of Zoology; P.F. Landrum, Southern Illinois University; M. Lydy, Southern Illinois University Carbondale / Fisheries and Illinois Aquaculture Center and Department of Zoology. Recent studies have determined that techniques, such as solid phase microextraction (SPME) fibers and Tenax beads, can predict bioaccumulation and potentially toxicity for several compounds and species. The objectives of the current study were to determine median lethal concentrations (LC50) and median effect concentrations (EC50) of the pyrethroid insecticide bifenthrin using bioavailability-based metrics, compare their ability to access toxicity to the use of whole sediment concentrations, as well as to make comparisons of the concentrations derived using each method in order to make assessments of accuracy and extrapolation potential. Four metrics were compared including solid phase microextraction (SPME) fiber concentration, pore water concentration derived using SPMEs, 6 h Tenax extractable concentration, and 24 h Tenax extractable concentration. Toxicity of bifenthrin was determined using two standard benthic species: *Hyalella azteca* and *Chironomus dilutus*. The variation in the LC50s and EC50s derived using bioavailability-based methods was comparable to variation among organic carbon normalized sediment concentrations, but improved over whole sediment concentrations. There was a relationship between SPME or Tenax and organic carbon normalized sediment concentrations. Additionally, there was a significant relationship between the SPME and Tenax concentrations across sediments. These correlations among metrics further demonstrate the relationship among these bioavailability-based endpoints. This study not only emphasizes the value of these techniques, but also provides usable benchmarks for implementation of these methods in environmental assessments.

**37 Combining bioavailability assays with modeling to predict PCBs in fish after remediation** H.F. Khoei, University of Maryland Baltimore County; U. Ghosh, University of Maryland Baltimore County / Civil & Environmental Engineering, University of Maryland Baltimore County / Department of Chemical, Biochemical, and Environmental Engineering, University of Maryland Baltimore County / Chemical, Biochemical, and Environmental Engineering; A. Place, A. Watson, Institute of Marine and Environmental Technology. While several in-situ (non-removal) remediation approaches for contaminated sediments have been explored, a central challenge with in-situ remedies is the length of time necessary to observe impacts, particularly in fish. An alternative is to make timely assessment of remediation progress by performing bioavailability measurements that indicate key pathways of exposure to fish and use these assessments to in conjunction with bioaccumulation models to predict uptake in the food chain. This talk will present initial results from a 3-year study that is evaluating in laboratory mesocosm experiments the feasibility of bioavailability assessments to predict uptake in fish and response to in-situ remediation. In order to develop a fundamental understanding of biouptake in fish and how that is impacted with changes in exposure pathways, a preliminary laboratory exposure study was performed using three types of sediment: clean sediments, PCB impacted sediments, and PCB impacted sediments treated with activated carbon (AC) to reduce PCB bioavailability. Replicate aquaria were arranged in a parallel flow configuration and fed with recirculating water. Polyoxymethylene passive samplers were placed in the sediment and water column in each aquarium. Zebrafish were deployed in each aquarium and were fed with algae based PCB-free food. Porewater PCB concentration

in PCB impacted sediment was high and was reduced by two orders of magnitude upon amendment with 5% activated carbon by weight. Porewater PCB concentration in AC-treated sediment was similar to that in the clean sediment aquaria. PCB in overlying water in the AC-treated sediment tanks were an order of magnitude lower than that in the untreated sediment tanks. PCB uptake in fish reflected PCB levels seen in the porewater and overlying water in each tank. Higher levels of PCBs in porewater corresponded to higher levels of accumulation in fish. These results are being incorporated in a PCB bioaccumulation model to allow prediction of PCB bioaccumulation in fish based on the assessment of changes in exposure as a result of in-situ treatment. Ongoing work is evaluating uptake in benthic invertebrates (freshwater oligochaetes) to evaluate benthic exposure pathway, and uptake in algae and daphnia to evaluate pelagic exposure pathway of PCBs to fish.

**38 Development of Stable Isotope Based Methods to Predict Bioavailability of Hydrophobic Organic Contaminants in Sediments** J. Gan, X. Cui, L. Delgado-Moreno, University of California Riverside; F. Jia, University of California, Riverside / Department of Environmental Sciences, UC Riverside / Department of Environmental Sciences; L. Bao, University of California Riverside / Chinese Academy of Sciences, University of California, Riverside / Department of Environmental Sciences; R. Lavado, University of California Riverside / Department of Environmental Sciences, University of California Riverside / Department of Environmental Science; D. Schlenk, University of California-Riverside / Department of Environmental Sciences; J. Gully, Environmental Scientist / Ocean Monitoring & Research Section, Supervising Environmental Scientist / Ocean Monitoring & Research Group; K.A. Maruya, Southern California Coastal Water Research Project, SCCWRP. Bioavailability of hydrophobic organic contaminants (HOCs) in impacted sediments is influenced by sediment properties (e.g., quantity and quality of organic carbon, black carbon), residence time, and other factors, making the bulk or total sediment concentration a poor predictor in risk assessment and management. Current methods for estimating the bioavailability of HOCs in sediments or soils suffer various theoretical or practical limitations. In this NIEHS project, we are exploring the use of stable isotope labeled reference compounds and the concept of isotope exchange to measure bioavailability in a versatile and reproducible manner. In the isotope exchange application,  $^{13}\text{C}$  or deuterium-labeled analogues are introduced into a sediment sample and mixed, and the aqueous phase obtained at the steady state by centrifugation or filtration is analyzed for the ratio of stable isotope labeled and native (non-labeled) concentrations, from which the exchangeability  $E$  of the native HOCs may be calculated. Preliminary results show that  $E$  is closely correlated with bioaccumulation of various HOCs into benthic invertebrates. In another application, stable isotope labeled standards are preloaded onto solid phase microextraction (SPME) fibers before placing the fiber into a sediment sample, and the exchange of labeled and non-labeled (native) HOCs on the fiber allows the estimation of absorption kinetics, enabling the estimation of freely dissolved concentration ( $C_{\text{free}}$ ) using short and flexible sampling times. Given that stable-isotope labeled standards are increasingly available, these methods are expected to be easily adoptable in most laboratories for investigation bioavailability of sediment-borne HOCs such as DDT, PCBs, PAH, and PBDEs, among others.

**39 Geochemical characteristics of the mine-impacted North Fork of Clear Creek and implications for stream recovery** J. Williamson, J. Ranville, Colorado School of Mines; J. Meyer, ARCADIS U.S. Inc; K. Dahl, S. Smith, N. Albuquerque, S. Johnson, Colorado School of Mines. Legacy acid generation, resulting from silver and gold mining wastes, has contaminated the North Fork of Clear Creek with high concentrations of metals such as aluminum, copper, iron, manganese and zinc. The U.S. Environmental Protection Agency (USEPA) has placed a high level of urgency on the remediation of this mine-impacted stream. In the fall of 2013, the USEPA will begin operating a new water treatment plant in the effort to remove the metals from the incoming point sources (Gregory Incline and National Tunnel) before they enter the stream. This presents an excellent pre- and post-remediation scenario in which metal-mixture toxicity and rate of aquatic geochemical recovery can be investigated in a natural water source. Elemental analysis of stream water samples are being performed to determine temporal and spatial variability of pre-remediation concentrations of metal contaminants; and laboratory experiments and geochemical speciation calculations are being performed to predict post-remediation in stream water chemistry. Concurrently, toxicity tests are being conducted with the

stream water and laboratory-prepared mimics of the stream water to help identify mechanisms of toxicity, support parallel modeling efforts and test whether single-metal biotic ligand models predict mixture toxicity well. The sediments, formed by metal oxide precipitation, may contribute metals to the water-column post-remediation. Metal-release rates are likely dependent on their physical and chemical characteristics. Experiments, performed at upstream locations, involving in-stream scouring/dissolution of metal-oxide coatings on rocks are being used to examine likely recovery rates.

**40 Monitoring PAH Bioavailability in Remediated Sediment Using Passive Sampling Devices** D. Shea, North Carolina State University / Department of Biology; K. O'Neal, X. Kong, X. Xia, P. Lazaro, W. Thorsen, North Carolina State University. The remediation of contaminated sediment is intended to either remove and or make unavailable the unacceptable exposure to sediment-bound chemicals. At a PAH-contaminated site near Tianjin, China we tested three remediation methods: removal of sediment, capping, and fortification with activated charcoal in replicate 1-m diameter microcosms and measured the PAH in caged bivalves and passive sampling devices (PSDs) deployed at the sediment-water interface and imbedded in the surface sediment. All three remediation methods dramatically decreased the dissolved PAH exposure in the surface sediment and overlying water. This decrease was reflected in much lower PAH concentrations in the bivalves and the PSDs. We used two different PSD polymers: polyoxymethylene (POM) and polyethylene (PE) and both gave equivalent results in post-remediation monitoring. The PE PSD had greater variability and higher PAH concentrations in the pre-treatment monitoring most likely due to the adherence of oil and/or coal tar product to the PE and not to the POM. There was also good agreement between PSDs and the bivalves, with greater differences observed in pre-treatment monitoring and with lower molecular weight PAH. Finally, we compared in-situ PSD data with ex-situ laboratory desorption experiments using the POM and again found good agreement. Our results indicate that both in-situ PSD deployment and the more easily measured ex-situ laboratory desorption experiments are adequate surrogates for measuring the bioavailable PAH in both baseline (pre-treatment) and post-remediation PAH contaminated sediment.

**41 Zebrafish as a model to address the impact of 17 $\alpha$  ethinylestradiol on fish health: a focus on down-regulated genes impacting innate immunity** J.D. Hansen, US Geological Survey-Western Fisheries Research Center, USGS WERC; J.C. Woodson, J.R. Winton, US Geological Survey-Western Fisheries Research Center; T.K. Bammler, R.P. Beyer, Department of Environmental and Occupational Health Sciences, University of Washington; E.P. Gallagher, Department of Environmental and Occupational Health Sciences, University of Washington / Department of Environmental and Occupational Health Sciences; D.E. Tillitt, US Geological Survey-Columbia Environmental Research Center. Environmental contaminants are believed to have profound impacts on fish health through modulation of immune responses. The innate immune response represents the first line of defense against microbial pathogens and is largely based upon the recognition and response to pathogen associated molecular patterns (PAMPs) through a range of pattern recognition receptors (PRRs) on cells of the innate immune system, leading to activation of inflammatory processes and the transition to adaptive immunity. To address the hypothesis that endocrine disrupting chemicals can lead to immune dysfunction in fish, zebrafish were sub-chronically exposed to environmentally relevant levels of 17 $\alpha$ -ethinylestradiol (EE2; 1ng/L and 10ng/L) and then injected with the microbial PAMPs, peptidoglycan and poly:IC to mimic infection. Tissues were then subjected to genome-wide microarray and pathway analyses, which revealed down regulation of key genes and pathways involved in innate immunity including pathogen recognition, complement activation and antigen presentation as verified by quantitative RT-PCR, Western blot and respiratory burst analyses of cells and tissues exposed to EE2. Taken together, our results strongly support the hypothesis that exposure to environmental concentrations of EE2 have profound impacts on fish health which would likely render fish more susceptible to pathogens.

**42 Gonadal gene expression profiles in Largemouth bass placed in organochlorine pesticide contaminated mesocosms surrounding Lake Apopka, Florida** C.J. Martyniuk, University of New Brunswick / Dept. of Biology/Canadian Rivers Institute, University of New Brunswick / Biology; M. Prucha, N. Doperalski, University of Florida; K.J. Kroll, University of Florida / Physiological Sciences; D. Barber, N. Denslow, University of

Florida. Lake Apopka (FL, USA) experienced heavy uses of organochlorine pesticides (OCPs) in the 1960s-1970 during an intense agricultural period and was declared a Superfund site by the US EPA. Aquatic organisms inhabit the freshwater systems in and around Lake Apopka, however the impacts of sub chronic exposure to OCPs in this natural environment are not known. We investigated the effects of the contamination on the reproductive axis of largemouth bass (LMB) (*Micropterus salmoides*), an apex predator, using a natural mesocosm. In October 2007, LMB were caught at DeLeon Springs (FL) and were stocked in the mesocosms, remaining there for 3 months before being sampled in January 2008 (early oogenesis). Additional fish were caught in February 2008 at DeLeon Springs, placed into mesocosms for 2 months, and sampled in April 2008 (oocyte maturation). LMB inhabiting the mesocosms for four months had a 2-20X higher contaminant load for OCPs (e.g. DDE, dieldrin, methoxychlor) than LMB collected at DeLeon Springs. GSI for fish collected in the mesocosm in April were not different compared to reference fish in early maturation phase. Vitellogenin levels in LMB collected from the mesocosms in January and April were not significantly different than fish collected from Welaka in late vitellogenin or early maturation respectively. Microarray analysis in the ovary revealed that the expression profiles of LMB found at reference sites were different compared to LMB in the mesocosms and formed a unique expression clade. Some notable transcripts that showed altered abundance in LMB from mesocosms were insulin-like growth factor I and steroidogenic acute regulatory protein. Interestingly, differentially expressed transcripts showed a significant and positive correlation for LMB sampled in January and April despite the 3 month period in between samplings. Sub-network enrichment analysis for cellular processes showed that retinoic acid metabolism and germ-cell development were decreased in mesocosm-exposed fish but processes such as vitellogenesis, amino acid catabolism, granulosa cell function, vitamin D metabolism, and hormone biosynthesis were increased in mesocosm-exposed fish. These data suggest that (1) LMB from the mesocosms are exhibiting unique gene profiles that may impair normal reproduction and that (2) microarray analysis in the field can provide site specific information by discriminating LMB from reference and polluted sites.

#### 43 Transcriptome analysis of BaP exposure in zebrafish embryos and larvae

**K.L. Willett**, University of Mississippi / Environmental Toxicology Research Program; **J. Corrales**, C. Thornton, University of Mississippi / Environmental Toxicology; **X. Fang**, Yale University; **L. Ballard**, B.E. Scheffler, USDA ARS. Polycyclic aromatic hydrocarbons (PAHs) are organic pollutants that arise from incomplete burning of carbon-containing materials, such as diesel, gas, petroleum, coal, wood, fat and tobacco. PAHs, like benzo(a)pyrene (BaP), are carcinogenic, mutagenic and teratogenic. Here we present differences in the transcriptome of developing zebrafish after a parental BaP waterborne exposure followed by an offspring exposure. Adult zebrafish (2 males x 4 females, N=3 replicate tanks per treatment) were exposed to control or  $42.0 \pm 1.9 \mu\text{g/L}$  BaP for 7 days. Eggs were collected and raised in normal conditions or continuously exposed to BaP until 3.3 and 96 hpf. Total RNA was extracted from a 30 embryo pool. Then, tagging and RNA-Seq libraries were constructed prior to Illumina HiSeq-2000 sequencing. To analyze data, RNA sequences were uploaded to galaxy <https://main.g2.bx.psu.edu/> and converted to FASTQsanger format with the "FASTQ Groomer" tool. RNA-seq reads were mapped to zebrafish genome (DanRer7) with the "Tophat for Illumina" tool in the galaxy. The resulting Binary Sequence Alignment/Map (BAM) files for each sample were merged into a single BAM file and imported to Partek Genomics Suite version 6.11. Differential expression on raw gene and transcript reads between treatments or time-points were analyzed with R package "EdgeR". Genes with false discovery rate (FDR) less than 0.05 were considered as genes with significantly altered expression. Functional ontology was conducted with the Database for Annotation, Visualization and Integrated Discovery (DAVID). Developmental differences between 3.3 and 96 hpf zebrafish were shown by 11823 differentially expressed genes and 12406 differentially expressed transcripts. BaP exposure resulted in 274 differentially expressed genes and 290 differentially expressed transcripts at 3.3 hpf and 930 genes and 885 transcripts at 96 hpf. The cluster with the highest enrichment score included functions such as gastrulation, cell migration, cell motility, localization of cell, embryonic morphogenesis, formation of primary germ layer and tissue morphogenesis. Supported by NIEHS R21ES019940.

**44 Genome-wide expression study reveals genetic determinants for the action of compounds as mixtures** **J.R. Shaw**, Dartmouth College / The School of Public and Environmental Affairs and The Center for Genomics and Bioinformatics; **S. Shankar**, Indiana University / The School of Informatics; **J.A. Lopez**, Indiana University / The Center for Genomics and Bioinformatics; **S.P. Glaholt**, Indiana University / The School of Public and Environmental Affairs and The Center for Genomics and Bioinformatics; **B.L. King**, Mount Desert Island Biological Laboratory; **J.K. Colbourne**, Indiana University / The Center for Genomics and Bioinformatics. At an annual "Environmental Genomics" summer course at the Mount Desert Island Biological Laboratory, participants learn how to design large scale experiments that are enabled by drastically increased sample-throughput. Experiments are conducted using 12-plex microarrays that are designed to study the freshwater microcrustacean *Daphnia* ([www.mdibl.org/courses/Environmental\\_Genomics](http://www.mdibl.org/courses/Environmental_Genomics)). We present a meta-analysis of two years of course data. For these experiments, two genetically and geographically unique *Daphnia pulex* isolates were exposed to incipient concentrations of six compounds (acetaminophen, arsenic, atrazine, cadmium, copper, nickel) and binary mixtures. The design includes four biological replicates per treatment, resulting in 200 comparative two-color hybridizations. For these analyses, signal data were normalized and fit to a linear model that evaluated main effects and interactions between contrasting variables of (1) every treatment and control, (2) binary mixtures and constituent compounds, and (3) genetic background. The analyses reveal that variation between genotypes is much greater than any treatment or treatment combination. Multi-dimensional scaling that reduces all variables along two axes segregates the response profiles by genotype along the first coordinate and by chemical class (metals vs non-metals) along the second. Interestingly, mixture response differed by genotype regardless of chemical class. One genotype produced additive response profiles for mixtures, while the other produced non-additive profiles that were either antagonistic or synergistic depending on mixture components. These results highlight the promise of environmental transcriptomics, while advising caution about the required scale of experiments needed to accurately interpret results. These studies contribute to and benefit from the *Daphnia* Genomics Consortium.

**45 Analysis of the Transcriptome of the Fathead Minnow to Determine Mechanisms of Toxic Action of Oil Sands Process Affected Water** **S.B. Wiseman**, University of Saskatchewan / Toxicology Centre, University of Saskatchewan / Post-Doctoral Fellow; **Y. He**, University of Saskatchewan / Toxicology Centre, University of Saskatchewan / PHD student; **P. Jones**, University of Saskatchewan / School of Environment and Sustainability, University of Saskatchewan / School of Environment and Sustainability and Toxicology Center; **M. Hecker**, University of Saskatchewan / Toxicology Centre; **J.W. Martin**, Trent University / Division of Analytical and Environmental Toxicology; **M.G. El-Din**, University of Alberta / Department of Civil and Environmental Engineering; **J.P. Giesy**, University of Saskatchewan / Biomedical Veterinary Sciences & Toxicology Centre, Michigan State University / Department of Animal Science, University of Saskatchewan / Toxicology Centre. Oil sands process-affected water (OSPW) is produced by the oil sands industry in North Eastern Alberta, Canada. This OSPW is a complex mixture of clay, residual bitumen, salts, metals, and dissolved organic compounds of which naphthenic acids are hypothesized to be the primary toxicant. Because OSPW is acutely and chronically toxic to aquatic organisms it must be stored in tailings ponds due to a policy of zero discharge. The mechanism(s) of toxicity of OSPW has received little attention. Because NAs are surfactants OSPW might be acutely toxic because of polar narcosis. However, due to the complexity of the mixture, it can be hypothesized that OSPW might exert its toxicity via multiple mechanisms. Understanding the toxicity of OSPW is required to effectively establish methods of assessing and predicting the toxicity of OSPW. Effects at the cellular, tissue, or whole organism level are often initiated at the molecular level, so investigation of the effects of exposure to OSPW on gene expression might provide insight into the mechanisms of toxicity of OSPW. The goal of this study was to use RNAseq to quantify changes in the abundances of transcripts in the livers of fathead minnows (*Pimephales promelas*) exposed to OSPW. A reference transcriptome was assembled from 877,829,873 paired-end and single-end reads generated from RNA isolated from livers of fathead minnows exposed to various stressors. Reads were *de novo* assembled into 62,103 contigs of 200bp or greater and annotation of the contigs against the NCBI non-redundant protein database identified 25,342 contigs with an e-value of  $\leq 10^{-5}$ . Mapping of paired-end reads generated from sequencing



RNA isolated from livers of sexually immature male fathead minnows exposed to freshwater or OSPW to the reference transcriptome resulted in significantly greater abundance of 109 transcripts and significantly lesser abundance of 95 transcripts in livers of fathead minnows exposed to OSPW. By assigning Gene Ontology (GO) terms the biological processes of “cellular processes”, “metabolic processes”, “biological regulation”, and “response to stimulus” were identified as being affected by OSPW. Analysis of GO terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) annotations identified oxidative metabolism, oxidative stress, apoptosis, and immune function as processes affected by OSPW, and which might explain some of the acute and chronic toxicity of OSPW.

#### 46 Gene Expression of Fathead Minnows Exposed to Municipal

**Wastewater Effluents** D. Vidal-Dorsch, Southern California Coastal Water Research Project / Toxicology, Southern California Research Project / Toxicology, Southern California Research Project; S.M. Bay, D. Greenstein, SCCWRP; C. Colli-Dula, University of Florida; L. Wiborg, City of San Diego Public Utilities Department, Environmental Monitoring and Technical Services Division; D. Petschauer, City of Los Angeles, Environmental Monitoring Division; N. Denslow, University of Florida. Although we can measure and detect the presence of contaminants of emerging concern (CECs) in wastewater effluents and receiving waters, we still do not have a good understanding of the ecological implications of their presence in aquatic environments. Effects caused by CECs may be subtle and diverse, comprehensive tools such as microarrays are necessary to identify toxicity mechanisms involved in these types of responses. In this study, we conducted laboratory exposures to investigate the use of gene microarray analysis in effluent exposures. We measured 32 CECs in effluents, plasma concentrations of vitellogenin (VTG), changes in secondary sexual characteristics, and performed microarray and pathway analyses in sexually mature male fathead minnows (*Pimephales promelas*). An Agilent, 8x15000, one color array was used. Fish were exposed for 14 days to control treatments and 5% concentrations of municipal wastewater effluents. Secondary and chemically enhanced advanced primary treated effluents were used. Males exposed to effluent had significantly higher concentrations of plasma VTG when compared to controls. Changes in secondary sexual characteristics (e.g., development of ovipositors) were observed in some fish exposed to effluent. Effluent exposure caused differential gene expression (vs. controls) in genes involved in several functions including, xenobiotic metabolism, estrogenicity and energy/metabolism processes, with different processes influenced by effluent type. Fish exposed to primary treated effluent had differentially expressed genes related to metabolic pathways (e.g., biosynthesis of cholesterol, aromatic acid metabolism, metabolism of estrogens and androgens). Exposure to secondary treated effluent affected genes involved in signaling pathways (e.g., regulation gap junction b, cell cycle regulation). Gene expression data provided valuable information regarding some of the mechanisms associated with effects observed at higher biological levels.

#### 47 Omic-based Effects Monitoring – Assessing Contribution of Treated Wastewater to Downstream Pollution

D. Martinovic, University of Saint Thomas, University of St. Thomas / Dept. of Biology, U.S. EPA, Mid-Continent Ecology Division / Mid-Continent Ecology Division, University of St. Thomas / Biology, University of Saint Thomas / Biology; A.C. Mehinto, University of Florida / Department of Physiological Sciences and Center for Environmental and Human Toxicology; N.D. Denslow, University of Florida / Department of Physiological Sciences and Center for Environmental and Human Toxicology, University of Florida / Dept. of Physiological Sciences & D.L. Villeneuve, U.S. EPA / National Health and Environmental Effects Research Laboratory, U.S. EPA; G.T. Ankley, US EPA / Office of Research and Development, National Health and Environmental Effects Research Laboratory, Mid-Continent Ecology Division, U.S. EPA / National Health and Environmental Effects Research Laboratory; H. Schoenfuss, St. Cloud State University / Aquatic Toxicology Laboratory, St. Cloud State University / Department of Biological Sciences WSB-273, St. Cloud State University / Department of Biological Sciences MS-273. The effects of wastewater treatment plant (WWTP) effluents on fish were studied at three Minnesota locations selected to represent a variety of treatment technologies, population sizes and geographic distributions. At each location, a mobile exposure laboratory trailer was set up to conduct 96-hr exposures of male fathead minnows to the wastewater effluent, and upstream and downstream receiving waters. Following the exposures, we conducted analyses of gene expression in the livers using a 15,000 gene microarray developed

specifically for fathead minnow. Gene ontology and gene set enrichment analysis (GSEA) were conducted. GSEA utilized gene sets corresponding to features represented within the gonad compartment of a conceptual model of the teleost brain-pituitary-gonadal axis (BPGA). Gene expression in fish was altered when they were exposed to WWTP effluents, or to the surface water collected either upstream or downstream of WWTPs. One of the prominent observations made in the microarray study was that the all effluents had an effect on genes that regulate reproduction (e.g., BPGA axis) and immune responses in fish. Overall, WWTP effluents had an effect on gene expression that was more similar to downstream sites than the upstream sites, suggesting a detectable effluent signature at all of the downstream sites. These findings suggest that WWTPs are an important contributor to the aquatic pollution, and that they have potential to adversely impact multiple physiological pathways important for organism's fitness (immune system functioning, reproductive success), which is consistent with the complex composition of WWTP effluents. We are currently integrating the results of this study with the apical organismal, metabolomic and chemical data to facilitate use of omic approaches for effects-based monitoring.

#### 48 Molecular insights into heavy metal tolerance in an extremophile polychaete

J. McQuillan, University of Exeter / Biosciences. Coastal and estuarine sediments are an effective sink for heavy metals from anthropogenic sources around river catchments and from the sea, driving the selection of tolerance traits amongst the endobenthic fauna. The best characterised example of an inherited tolerance to heavy metals in animals is considered to be the population of *Nereis (Hediste) diversicolor* (Harbour Ragworm) within Restronguet Creek, a branch of the Fal estuary, Cornwall, UK. The sediments within the upper reaches of Restronguet creek are burdened with extreme levels of Cu, Zn, As, Cd, Mn and Fe; and restrict macro and meiofaunal communities, with the exception of the local population of *Nereis diversicolor*. The Restronguet Creek population is significantly more resilient to metal cations than populations from elsewhere within the Fal estuary, and accumulate significantly higher levels of heavy metals within their tissues, yet little is known about the molecular mechanisms underlying tolerance. Understanding how estuarine organisms adapt and survive under such circumstances is of paramount importance if we are to forecast and manage the impact of global change in these and other marine systems. To elucidate the molecular basis for metal resistance in *N. diversicolor* we utilised 454 sequencing to generate a transcriptome from normalised cDNA libraries, and identified unique transcripts with homology to metal handling and detoxification-associated genes in model organisms. The regulation of select copper-associated gene transcripts was measured in *Nereis diversicolor* from Restronguet Creek and compared with animals from adjacent sites within the Fal estuary having intermediate and low levels of metal contamination. The results indicate that the Restronguet Creek population undergo regulatory changes at the transcriptome level, implying a mechanism for heavy metal detoxification based upon metal cation binding and sequestration, compartmentalisation and storage, and efflux.

#### 49 Canary in a coal mine: Marine mammals as indicators of contamination by persistent organic pollutants (POPs) in the Pacific Ocean

P.S. Ross, Fisheries and Oceans Canada / Institute of Ocean Sciences. A variety of abiotic and biotic matrices have been used in studies documenting trends in Persistent Organic Pollutants (POPs) in the aquatic environment and effects on the health of organisms. Monitoring of effluent discharges, sediments and sessile species have pinpointed contaminant sources and informed management on the effectiveness of regulations on anthropogenic emissions. However, such focussed efforts provide an incomplete picture of the implications of POP contamination for aquatic food webs, habitat, and health of biota. Marine mammals have long provided an integrated overview of POP contamination of aquatic food webs. In the NE Pacific Ocean, our research has centered on killer whales (*Orcinus orca*), which are among the most PCB-contaminated marine mammals in the world. We recently reported significant relationships between PCBs and the upregulation of five gene transcripts, including Aryl hydrocarbon, thyroid, estrogen, metallothionein and interleukin-10 receptors. We have also shown that while ‘local’ sources explain some of PCBs in killer whales, ‘global’ or trans-Pacific sources also contribute. Unlike killer whales, harbour seals (*Phoca vitulina*) provide a more regional source signal, as they are non-migratory and consume primarily resident fish species. Harbour seal samples reveal a steady decline in PCBs in coastal food webs until recently, but an exponential increase in PBDEs until 2005. Research on these two marine mammals indicates that

regulations and international treaties can lead to positive outcomes in upper trophic level marine mammals. However, we suggest that more precaution is needed in the regulation of chemicals deemed to be persistent and bioaccumulative; modelling suggests that killer whales will not be 'protected' from PCB-related health risks until the end of the 21<sup>st</sup> century. The interpretive strength of studies in marine mammal ecotoxicology is clearly improved when such factors as age, sex, feeding ecology, distribution, and metabolism are incorporated into study designs and data interpretation. Marine mammals can serve as informative 'sentinels' of food web contamination when such biological and ecological features are carefully considered.

#### **50 Persistent Organic Pollutants (POPs) in the Puget Sound Ecosystem: An evaluation of POPs in fecal samples of Southern Resident killer whales**

**J.I. Lundin**, University of Washington, Center for Conservation Biology; D. Boyd, NOAA, Northwest Fisheries Science Center; K.A. Parsons, NOAA, Northwest Fisheries Science Center and Alaska Fisheries Science Center; B. Anulacion, NOAA, Northwest Fisheries Science Center; R.K. Booth, University of Washington, Center for Conservation Biology; G.M. Ylitalo, NOAA, Northwest Fisheries Science Center; S.K. Wasser, University of Washington, Center for Conservation Biology. The Puget Sound ecosystem of Washington State has been riddled with human impacts. Exposure to persistent organic pollutants (POPs) has been listed as a primary risk factor for the endangered Southern Resident killer whale (SRKWs, *Orcinus orca*) population that resides in these waters. The objective of this study was to evaluate POPs (PBDEs, PCBs, DDTs, and other chlorinated pesticides) associated with endocrine disruption in SRKW scat (feces) and to describe POP contamination level by pod, age, sex and prey availability. Samples were collected using highly trained detection dogs that ride on the bow of a boat and use scent to locate floating killer whale scat. Using this non-invasive method we collected more than 90 samples across our 5 month study period, allowing an evaluation of temporal patterns of toxicant excretion in killer whale scat relative to prey availability and other environmental pressures. Laboratory analyses of the samples include contaminant quantification using gas chromatography/mass spectrometry, DNA confirmation of species, sex, and individual identity, and reproductive and thyroid hormones measurements by radioimmunoassay. Scat samples analyzed to date demonstrate a congener profile of PCBs similar to the profile found in blubber samples of the same population. This is particularly the case early in the season, when prey abundance is relatively low; specifically, the proportion of PCB138, 149, and 153 (6 chlorine PCBs) in the scat closely match the pattern found in blubber samples during that time. Scat collected during the peak Chinook salmon run tend to contain a disproportionate amount of the lesser chlorinated (< 6 chlorines) PCBs compared to blubber. Pod, sex and age pod differences in fecal POP levels are also consistent with expectations based on measures in blubber biopsy samples. The toxicant profiles in the scat demonstrate significantly higher  $\Sigma DDT/\Sigma PCB$  ratios in K and L pods, known to forage off the California coast, compared to J pod ( $p=0.05$ ), and  $\Sigma PCB$  and  $\Sigma PBDE$  levels are lower in adult females compared to the level in an adult male (J pod, age 19) for the samples analyzed thus far. The study is ongoing with the ultimate goal of evaluating population- and individual-based models that associate temporal contaminant levels with endocrine disruption and adverse reproductive health outcomes.

#### **51 Towards PBDE sediment quality guidelines that are protective of killer whales in British Columbia, Canada: insight from foodweb bioaccumulation models**

**J. Alava**, Simon Fraser University / School of Resource and Environmental Management (Faculty of Environment), Fundacion Ecuatoriana para el Estudio de Mamíferos Marinos (FEMM) / FEMM Filial Playas, Simon Fraser University / School of Resource and Environmental Management, Faculty of Environment; P.S. Ross, Fisheries and Oceans Canada / Institute of Ocean Sciences. At the top of the marine food web, resident killer whales in the coastal waters of British Columbia are heavily contaminated with persistent organic pollutants (POPs), including polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs). The northern and southern populations of resident killer whales are listed, respectively, as threatened and endangered under the Canadian *Species at Risk Act* (SARA), which protects species at risk from being killed or harmed and protects any part of their Critical Habitat from destruction. The Resident Killer Whale Recovery Strategy identified contaminants, reduced prey and disturbance (noise and physical) as conservation threats to population recovery. Sediments in areas and Critical Habitat of resident killer whales contain complex mixtures of contaminants, and material intended

to be dredged, resuspended and disposed of at sea is screened for a select list of contaminants. Because killer whales are long-lived and occupy a very high trophic level, they are at particular risk to accumulating high concentrations of not only PCBs, but PBDEs and several other related compounds. In order to assess the impact of PBDE contaminated sediments and given the complexity of killer whale ecology and that of their primary prey (Chinook salmon), we developed a novel food web modeling tool for the Strait of Georgia, BC, Canada. The modeling approach is based on the distribution of PBDEs among sediments, the water column, and biota, and predicts concentrations that will accumulate in animals throughout a lifetime of exposure. We predicted that that PBDE concentrations in both populations of resident killer whales exceed between 32 and 100% three health effect thresholds for PCBs established for marine mammals (i.e. 1.3, 10, and 17 mg/kg, lipid weight) should their prey be exposed throughout their lives to PBDE concentrations in sediments that are equivalent to the CCME ISQG, the CEPA Action Level Low and the BCMWLAP Sediment Quality Criteria for PCBs. We derived a preliminary sediment PBDE concentration that would protect 95% of resident killer whales of 1.0  $\mu\text{g/kg}$  dry weight. This newly developed food web model can be employed as a risk management tool in support of endangered species protections for killer whales.

#### **52 Persistent Pollutants in Paradise: What stranded Hawaiian cetaceans can tell us about biomarkers in the context of multiple stressors and disease**

**B. Jensen**, Hawai'i Pacific University / Natural sciences. Understanding the factors that tip the balance toward mortality and morbidity in vulnerable cetacean populations is a primary concern in marine mammal toxicology. We have recently identified several diseases of concern in stranded Hawaiian cetaceans, including *Cryptococcus*, *Brucella*, herpesvirus, and most recently (and troublingly), cetacean morbillivirus. The aryl hydrocarbon receptor (AHR) signal transduction pathway is well known to mediate the effects of planar halogenated aromatic subsets of persistent organic pollutants (POPs), resulting in cytochrome P450 1A1 (CYP1A1) induction, carcinogenesis, endocrine disruption, and immunosuppression. This pathway has been the focus of our initial work in understanding POP effects in Hawaiian cetaceans. Unlike regions that have a few dominant cetacean species, Hawaii claims 19 "common" cetacean species, and nearly all are high trophic level odontocetes. Work with this sample archive requires a multispecies approach to understand how certain biomarkers can (and cannot) be used toward inferring health status. Despite their geographically remote habitat in the central North Pacific, Hawaiian cetaceans have organic and trace element contaminant burdens comparable to cetaceans residing near continental coastlines. We assessed the species and inter-individual variability in the AHR, a biomarker for susceptibility, and found that the ligand binding domain of the AHR is highly conserved across 13 species with very low polymorphism, suggesting that odontocetes in general share the previously established potential for high sensitivity to AHR-mediated effects. Next, we assessed the species and tissue-specific expression of CYP1A1 using immunohistochemistry, western blotting, and real time PCR. These results suggest an extremely dynamic state of contaminant distribution and effect across tissue compartments at critical stages of disease progression. Several lines of evidence suggest that persistent POPs as well as non-persistent agonists activate the AHR pathway. Our work on stranded Hawaiian cetaceans is allowing us to build a case for the use of biomarkers for health assessment and to better understand relationships to life history and physiological parameters. Despite the number of confounding factors that stranded animals bring to toxicological analyses, contaminant impacts in health-compromised animals continue to be our primary conservation concern.

#### **53 Persistent organic pollutant concentrations in cetaceans stranded in the Hawaiian Islands, 1997 – 2011**

**M.J. Bachman**, Hawai'i Pacific University / Natural sciences; J.M. Keller, National Institute of Standards and Technology (NIST) / Analytical Chemistry Division; K. West, Hawai'i Pacific University; B. Jensen, Hawai'i Pacific University / Natural sciences. Persistent organic pollutants (POPs) are man-made chemicals that bioaccumulate, persist in the environment for long time periods, and are toxic. Many studies have determined concentrations of POPs in sentinel species, such as cetaceans (dolphins and whales), but no studies have quantified POPs in stranded cetaceans within the last 30 years around the Hawaiian Islands. A suite of 132 POPs were measured in the blubber of 16 species of cetaceans that stranded in Hawai'i from 1997 to 2011. The sample set represents both toothed dolphins and whales ( $n=33$ ) and baleen whales ( $n=3$ ) with additional samples pending analysis ( $n=6$ ). Detectable contaminants



included from highest to lowest general concentration are dichlorodiphenyltrichloroethanes (DDTs), polychlorinated biphenyls (PCBs), chlordanes, toxaphenes, polybrominated diphenyl ethers (PBDEs), hexachlorobenzene (HCB), mirex, hexachlorocyclohexanes (HCHs), hexabromocyclododecanes (HBCDs) and octachlorostyrene. Preliminary medians (standard deviation) in ng/g lipid are: 9,070 (24,400) for  $\Sigma$ DDTs, 5,280 (18,000) for  $\Sigma$ PCBs, 868 (2,230) for  $\Sigma$ chlordanes, 864 (1,690) for  $\Sigma$ toxaphenes, 197 (1,710) for  $\Sigma$ PBDEs, 186 (207) for HCB, 152 (951) for mirex, 99.0 (84.7) for  $\Sigma$ HCHs, 33.9 (178) for  $\Sigma$ HBCDs, and 2.36 (2.66) for octachlorostyrene. Significant species differences were observed for all compound classes. Calves were significantly lower than juvenile, subadult, and adult age classes for  $\Sigma$ PCBs,  $\Sigma$ DDTs, and  $\Sigma$ chlordanes. Decreasing temporal trends were evident for  $\Sigma$ HBCDs and mirex. This presentation will also compare these concentrations to other marine mammal POP data on a global scale as well as against threshold values previously determined for marine mammal toxicity.

#### 54 Methylmercury metabolism across six Arctic marine mammals

– **Interspecies differences in exposure and neurotoxic risk** N. Basu, University of Michigan / Department of Environmental Health Sciences, University of Michigan / Department of Environmental Health; D. Nam, Chonnam University; J. Rutkiewicz, University of Michigan; C. Sonne, R. Dietz, University of Aarhus; T. Beschoft, Aarhus University; P. Leifsson, Royal Veterinary and Agricultural University. Arctic marine mammals bioaccumulate some of the highest methylmercury burdens of any species, though little is known about internal metabolic processes and ultimately adverse outcomes and inter-species sensitivities. Here we performed a comparative study whereby liver and brain tissue was studied from polar bears, pilot whales, harbor porpoises, narwhal, Baikal seal, and ringed seal. For most animals, the brain was dissected into several regions of functional significance (frontal cortex, temporal cortex, occipital cortex, basal ganglia, brain stem, cerebellum, hippocampus, hypothalamus, thalamus, and pituitary). In all tissues, total and organic (methyl) mercury were determined. In some cases selenium (which binds to mercury in a 1:1 molar ratio) was measured, in addition to other persistent contaminants (e.g., PCBs, PBDEs). To explore whether exposures were of neurological concern, a suite of neurochemical biomarkers (e.g., NMDA receptor) was measured across the brain regions and then related back to the measured contaminants. Several results are noteworthy and highlight tremendous inter-species variability in exposure and risk. Across species, the highest mercury levels were generally found in the pituitary tissue. While in most brain regions organic mercury was >80% of total mercury, in the pituitary this value was only ~50%. Brain mercury content was much greater in pilot whales, narwhal, and harbor porpoises (many exceeded 10ppm) versus polar bear, ringed seal, and Baikal seal (few exceeded 1ppm). Changes in some neurochemical biomarkers could be related to brain mercury levels in the aforementioned species with the highest mercury exposures. In a sub-study of polar bears, PCBs and PBDEs did not relate to any neurochemical biomarker. Differences in selenium handling and mercury demethylation are also apparent across species. Overall, these results suggest that certain marine mammals (pilot whales, harbor porpoise) accumulate mercury at levels capable of causing subclinical neurological effect, but others (e.g., polar bears) may have developed unique schemes to sequester or eliminate mercury and thus limit brain exposures.

#### 55 Health risks in ringed seals associated with a point source release of PCBs at a military station in Arctic Canada

T.M. Brown, / Department of Biochemistry and Microbiology, Royal Military College of Canada / Environmental Sciences Group, University of Victoria, Institute of Ocean Sciences; P.S. Ross, Fisheries and Oceans Canada / Institute of Ocean Sciences; C. Helbing, N. Veldhoen, University of Victoria; A. Fisk, University of Windsor; K. Reimer, Royal Military College of Canada. Despite the widespread contamination of aquatic food-webs by polychlorinated biphenyls (PCBs), few studies have unequivocally demonstrated adverse effects specifically attributed to this industrial chemical on marine mammals. Causal evidence linking PCBs to toxic effects in free-ranging marine mammals is generally confounded by the highly complex contaminant mixtures to which they are exposed. Elevated levels of PCBs have been measured in ringed seals (*Pusa hispida*) from the north Labrador coast. Although long-range transport of PCBs through air and ocean currents deliver PCBs and related compounds to Labrador, local sources of PCBs from military facilities along the Labrador coast also exist. Saglek, a former 'Pole Vault' military

radar station, is one of two sites where PCB releases have contaminated the adjacent marine environment. Approximately 25% of ringed seals sampled from 2009-2011 had levels of PCBs that exceed health effects thresholds for another pinniped species, the harbour seal (*Phoca vitulina*). In order to evaluate the implications for ringed seal health, we developed quantitative real-time PCR assay to evaluate the mRNA expression of contaminant-sensitive endpoints, including transcripts encoding the aryl hydrocarbon, thyroid hormone, estrogen, and retinoic acid receptors. Preliminary results show that higher mRNA expression of the AhR receptor and thyroid receptor are correlated with increasing levels of PCBs. The combination of gene expression endpoints, contaminant profiles, and insight into habitat use and feeding ecology afforded us a unique opportunity to explore source, transport, fate of PCBs in a coastal area, and assess the effects of PCBs on the health of ringed seals. Results are relevant not only in terms of the health of the ringed seal population, but also the local Inuit who rely heavily on seals and fish for their subsistence.

#### 56 Are PBT evaluation and environmental criteria protecting marine mammals?

EA Gobas, Simon Fraser University / School of Resource & Environmental Management, Faculty of Environment. Fifty years after Rachel Carson's book *Silent Spring* revealed publicly the potential devastating effects of commercial pesticides on wildlife, many countries including the members of the EU, the US, Canada, Australia and Japan have developed regulations for screening commercial chemicals for their persistence (P), bioaccumulation (B) and toxicological (T) properties. But are we any closer to protecting the wildlife species that Rachel Carson was alerting us about? This presentation discusses the current regulatory methods and risk assessment methods that are used to date to assess the environmental in the context of their ability to adequately protect marine mammals and other high trophic level organisms from harmful environmental pollutants. The presentation evaluates criteria for bioaccumulation and toxicity, water and sediment quality guidelines, tissue consumption criteria for fish consuming wildlife and methods for ecological risk assessment. It is concluded that current methods for the evaluation and management of chemical pollutants are not adequate and appropriate for the protection of marine mammals. Recommendations for further improvement of international regulations and management practices are presented.

#### 57 Sources of Pyrethroids in Municipal Wastewater

K.D. Moran, TDC Environmental, LLC; P.L. TenBrook, U.S. Environmental Protection Agency, Region IX. Pyrethroid insecticides have been linked to widespread toxicity in California surface waters. Published and unpublished studies have also linked pyrethroids in municipal wastewater treatment plant effluent to toxicity to *Hyaella azteca* and *Americamysis bahia*. Recently, researchers and municipal wastewater treatment plants have developed analytical methods capable of measuring nanogram per liter concentrations of pyrethroids in municipal wastewater effluent. Initial studies applying these new methods reported detection of bifenthrin, cyfluthrin, cypermethrin, lambda-cyhalothrin, and permethrin in effluents, sometimes at concentrations higher than potential water quality criteria developed by U.C. Davis for the Central Valley Water Board. Most of the pyrethroids detected in municipal wastewater do not have obvious pathways for discharge to sewer systems. To address this information gap, a conceptual model was developed to describe the sources and pathways for pyrethroids discharges to municipal wastewater treatment plants. This model is based on a review of scientific and engineering literature, pesticide product labels, previously identified discharge pathways for other pollutants, and unpublished data from municipal wastewater treatment plants. The conceptual model categorizes urban pesticide use patterns and disposal practices, and identifies pathways linking pesticide applications with sewer discharges. The model assumes that the bulk of pesticide applications are made to sites specified on product labels, but considers both legal and illegal disposal practices. The model was developed to serve as a tool to prioritize data collection to characterize potential sewer discharge sources of pyrethroids. Understanding pyrethroids sources is critical for development of targeted risk management actions to respond to the pyrethroid-related compliance challenges facing municipal wastewater treatment plants.

#### 58 Fate of pyrethroids through wastewater treatment processes: Bench-scale and full-scale evaluations

J. McGrath, HDR Inc; D. Tessier, A. Barefoot, DuPont Crop Protection; J. Cleary, HDR Inc; H. Monteith, Hydromantis; K. Ohlinger, Sacramento Regional Wastewater Treatment Plant;

H. Ramil, Sacramento Regional Wastewater Facility. The fate of pyrethroids through typical wastewater treatment processes was evaluated through laboratory bench-scale testing and full-scale wastewater plant sampling. At the bench-scale level, removal of pyrethroids through primary settling, anaerobic and aerobic biological treatment and ultra-filtration was investigated. Pyrethroid concentration data were used to quantify removal via sorption and degradation in each process and pyrethroid concentrations exiting the system. Removals achieved depended on the pyrethroid. Similar removals were observed via anaerobic and aerobic biological degradation ranging from approximately 32 to 81%. In the aerobic system, sorption accounted for an additional 4 to 14% removal. Filtration was shown to significantly reduce the concentration of pyrethroids in the simulated effluent. Full-scale wastewater plant sampling was conducted at two facilities, located in California and in New York. At each facility, sampling of influent, effluent, sludges and intra-process locations was conducted. Each facility was modeled using TOXCHEM+, a commercially available program that predicts the fate of trace organic contaminants through wastewater collection and treatment processes using mass transfer and mass balance principles. From the modeling effort, the physical chemical properties describing the fate of the pyrethroids were determined as well as biodegradation rates. Generally, sorption to biosolids and biodegradation were the major removal mechanisms of the pyrethroids accounting for more than 93% removal of the total pyrethroid. Sorption and biodegradation appeared to be competing removal mechanisms. At the New York facility, sorption and biodegradation removals were on average 65 and 30%, respectively. At the California facility, biodegradation removals were higher and averaged 56% and sorption removals were on average 37%. Volatilization losses were negligible for most of the pyrethroids. For both facilities, 2 to 6 % of the input mass of pyrethroids was discharged via the effluent.

**59 Pyrethroid pesticides in municipal wastewater: sources, treatment effectiveness, and effluent quality** D. Weston, University of California / Department of Integrative Biology; H. Ramil, Sacramento Regional Wastewater Treatment Plant; M. Lydy, Southern Illinois University. Pyrethroid insecticides can enter municipal wastewater through drain disposal or urban runoff, but little published information is available on their presence in wastewater in the United States, or their extent of removal during treatment. Influent and effluent of the treatment plant serving communities surrounding Sacramento, California were sampled for pyrethroid insecticides for approximately one year. The compounds were in influent at a total concentration of 200-500 ng/L, with permethrin, bifenthrin, cypermethrin, and lambda-cyhalothrin commonly detected. Sampling within the wastewater collection system suggested the pyrethroids often originated from drain disposal, not entry of urban runoff into the system, and that wastewater from residential areas was similar in pyrethroid composition to the larger metropolitan area as a whole. Secondary wastewater treatment removed approximately 90% of pyrethroids, but those remaining in final effluent still exceeded concentrations acutely toxic to sensitive species. Toxicity was consistently evident in the final effluent. The extent of toxicity in receiving waters will be site-specific, but the large river into which this particular plant discharged provided sufficient dilution such that only slight toxicity of unknown cause was found in one sample.

**60 Distribution of the pyrethroid insecticide concentration between freely dissolved and particle-bound forms in dechlorinated secondary effluent** E. Parry, University of California, Davis / Agricultural and Environmental Chemistry, University of California, Davis / Agricultural and Environmental Chemistry Graduate Group, University of California, Davis; T.M. Young, University of California-Davis / Dept. of Civil & Environ. Engineering. Pyrethroid insecticides are a major current use insecticide class in the US. Pyrethroids have been detected in the dechlorinated final effluent (DFE) of Municipal Wastewater Treatment Facilities at levels toxic to aquatic arthropods. Pyrethroids could pass through the treatment process in a variety of forms: freely dissolved, complexed with organic matter, and adsorbed to sub-micron particles. Effluent from a secondary treatment system was sampled six times in the course of a yearlong study. The DFE was fractionated by laboratory centrifugation to separate different particle size cuts. The pyrethroid concentration associated with each fraction was quantified. Solid phase microextraction (SPME) was used with pyrethroid serial additions to measure an organic carbon normalized distribution coefficient ( $K_{oc}$ ) between pyrethroids and the suspended wastewater particulate matter. Values for log  $K_{oc}$  ranged between 6.5-7.4 depending on sampling event and

chemical. Calculation with the measured  $K_{oc}$  and whole water pyrethroid concentration predicts that < 5% of the pyrethroid is in the freely dissolved phase. The sorbed portion was subdivided by particle size based upon the particle fractionation. The form of the chemical in the effluent has implications for removal mechanisms, bioavailability and fate and transport.

**61 Pesticide Detections and Toxicity in Urban Runoff from Neighborhoods in Northern and Southern California** M.P. Enslinger, R. Budd, California EPA / California Department of Pesticide Regulation; K.S. Goh, California EPA / Department of Pesticide Regulation; L. Deanovic, S. Teh, University California, Davis / Aquatic Health Program. Since 2008 the California Department of Pesticide Regulation has conducted monitoring studies to determine pesticide content in urban runoff. Recently, toxicity testing has been added to this monitoring. Water samples from 11 sites in northern and southern California collected during rainstorm and dry season monitoring were analyzed for pesticides and tested on *Hyalella azteca*. Sediment samples with concomitant sediment toxicity were analyzed from some of the sites. Water samples collected during rainstorm events contained an average of nine pesticides per sample; none of the *H. azteca* survived in the toxicity tests. Based on benchmarks or water quality criteria, bifenthrin, fipronil, cyfluthrin, cypermethrin, and malathion likely contributed to the toxicity. Samples also contained 2,4-D, dicamba, triclopyr, desulfinyl fipronil, imidacloprid, and permethrin; the combined effects of all the detected pesticides as well as other environmental stressors may have impacted toxicity. Water samples collected during dry season monitoring have fewer pesticides and likely affect toxicity accordingly. Sediment samples were toxic to *H. azteca*. Bifenthrin was the most commonly detected pyrethroid and gave the highest toxicity units; lambda-cyhalothrin, cyfluthrin, and cypermethrin were also implicated in sediment toxicity.

**62 Trends in Stream Contaminants and Toxicity in California: The SPoT Program** K. Siegler, University of California – Davis / Environmental Toxicology; B.M. Phillips, University of California – Davis / Environmental Toxicology, University of California-Davis / Granite Canyon; B. Anderson, University of California – Davis / Department of Environmental Toxicology; J. Voorhees, University of California – Davis / MPSSL – Environmental Toxicology; L. Jennings, R. Tjeerdema, University of California – Davis / Environmental Toxicology. The Stream Pollution Trends (SPoT) program is a statewide monitoring program under the umbrella of the Surface Water Ambient Monitoring Program (SWAMP). SPoT is designed to detect trends in contamination and toxicity in major watersheds of California. Sites at the base of 100 watersheds were selected for integrative measurements of sediment toxicity and a suite of pesticides, trace metals, and industrial compounds. Toxicity was observed at 22% (2008), 35% (2009), 25% (2010), and 19% (2011) of the sites using the 10d *Hyalella azteca* test. The prevalence of pyrethroid pesticide detections increased from 55% in 2008 to 76% in 2010, with maximum concentrations of sum pyrethroids increasing from 113.0 ng/g to 1043.1 ng/g. In 2010 and 2011, a subset of sites was tested for toxicity at 15°C, as well as the standard test temperature of 23°C. Within this subset, the percent of sites that were toxic increased from 35%, when tested at 23°C, to 62% when tested at 15°C. This suggests pyrethroid pesticides contributed to the observed toxicity. Land use in each watershed was characterized using data from the National Land Cover Database and Spearman multivariate correlations were conducted to determine correlations between land use type, sediment toxicity, and contaminant concentrations. In 2008, pyrethroid pesticides were significantly correlated with urban watersheds. The overall trends suggest that sediment toxicity is fairly consistent, pyrethroid detections are increasing, and detections of pyrethroid pesticides are more strongly correlated with urbanized watersheds.

**63 Pesticides, Urban Streams, and Pacific Salmon: What Have We Learned Over the Last 10 Years** C. Grue, Washington Cooperative Fish and Wildlife Research Unit, University of Washington / School of Aquatic and Fishery Sciences, USGS, Washington Cooperative Fish and Wildlife Research Unit / University of Washington; K.A. King. Pesticides are frequently detected in urban streams and are believed to be primarily the result of homeowner use with concentrations often exceeding those reported in surface waters associated with agricultural areas. The listing of several runs of Pacific salmon as threatened or endangered and associated federal, state, and local efforts to restore/enhance salmon habitat in the Pacific Northwest make it imperative that the factors associated with these population declines are understood. We review the results of laboratory studies conducted over

the last 10 years that have examined direct toxic effects on most aspects of the life cycle of Pacific salmon using primarily juvenile coho (*Oncorhynchus kisutch*) and surrogate rainbow trout (*O. mykiss*). Exposures include single active ingredients, formulated products, or mixtures ("cocktails") representative of those most frequently reported in urban streams in western Washington State. Endpoints include brain acetylcholinesterase (AChE) activity, pre-spawn survival, growth and survival during early life stages, smoltification, olfaction, immune competence, and return rates of adults exposed to pesticides as juveniles. Results of studies by the authors as well as others will be included. Exposures will be compared to data available on environmental concentrations of pesticides within surface waters in both urban and agricultural areas. Data gaps will be identified. We conclude with directions for future research.

**64 Occurrence and toxicity of current-use pesticides in sediment from an urban stream in Guangzhou, China** H. Li, Guangzhou Institute of Geochemistry / Chinese Academy of Sciences, Graduate School of the Chinese Academy of Sciences; B. Sun, Guangzhou Institute of Geochemistry, Chinese Academy of Sciences; J. You, Guangzhou Institute of Geochemistry, Chinese Academy of Sciences / State Key Laboratory of Organic Geochemistry. Non-point source runoff greatly contributed to pesticide contamination in urban aquatic ecosystems, and the contribution increased with the reduction of contribution from the wastewater plant effluents due to the improvement of sewage treatment capability in the cities. Research on environmental occurrences and ecotoxicological significance of current-use pesticides (CUPs), however, was limited in China. In the present study, sixteen sediments were collected from an urban stream in a Chinese city, Guangzhou. Four classes of CUPs including organophosphates (OPs), pyrethroids, fipronil and abamectin, were analyzed. Chlorpyrifos was the only OPs detected above the reporting limit, while pyrethroids, especially cypermethrin, were the most dominant pesticides in the sediments, with a mean concentration being 140 ng/g dry weight (dw). Additionally, fipronil and its two metabolites, fipronil sulfide and fipronil sulfone, were also detected in all sediments with a mean concentration being 13.7 ng/g dw. Elevated concentrations of abamectin were also detected with a mean concentration of 12.6 ng/g dw. On the other hand, sediment toxicity was evaluated using *Chironomus dilutus* and 15 of 16 sediments collected from the stream exhibited acute toxicity, within which 81% of sediment samples caused 100% mortality. Abamectin, fipronil and pyrethroids (mainly cypermethrin), were regarded as the principal contributors to the noted toxicity to the midges, with average predict toxic units being 2.44, 2.13, and 1.88, respectively. Results also showed that sediments in the downstream of the stream, where residential and industrial regions were located, had elevated CUP concentrations and sediment toxicity. Principal component analysis indicated that chlorpyrifos, fipronil and abamectin mainly originated from agricultural use in the urban villages, whereas pyrethroids were mainly released from residential use. As the first study to link sediment residues of fipronil and abamectin with toxicity to non-target species in urban streams in China, the present study urged more research on ecotoxicological effects of CUPs in order to catch up the rapid changes in pesticide application patterns in China.

**65 Change in Inputs to Coastal Waters off Southern California in the Forty Years Since Adoption of the Federal Clean Water Act** G. McGowen, City of Los Angeles / Environmental Monitoring Division. The southern California coast is an economic engine for the region, drawing in both residents and visitors. It supports a multitude of industries, businesses, and jobs, as well as recreation. Like developed coastlines around the world, the coastal waters off southern California also serve as a disposal site for the residues of human life and activities, including stormwater runoff and treated wastewater. By the 1960s, the conventional wisdom about the effects of wastes in the environment was being increasingly called into question as adverse impacts became increasingly obvious. The result was a flurry of legislative activity between 1960 and 1980, including the Federal Clean Water Act in 1972. In the forty years since enactment of the Federal Clean Water Act, a lot of resources have been expended to comply with it. Was anything accomplished? Was it worth it? How have things changed? The Technical Advisory Group to the Commission of the Southern California Coastal Water Research Project recently undertook a collaborative effort to assess the impact of the Federal Clean Water Act on southern California Coastal waters. This presentation addressed one aspect of this: changes in what we put into southern California Coastal waters.

**66 Trends and factors affecting coastal hypoxia in the Southern California Bight (SCB)** J.T. Booth, UCLA / Department of Atmospheric and Oceanic Sciences; C. Woodson, Stanford University / Department of Civil and Environmental Engineering; F. Micheli, Stanford University / Hopkins Marine Station; L. Crowder, Center For Ocean Solutions; E. Hazen, National Oceanographic and Atmospheric Administration / Pacific Fisheries Environmental Laboratory; E. McPhee-Shaw, Moss Landing Marine Laboratory; S.B. Weisberg, Southern California Coastal Water Research Project; M. Sutula, Southern California Coastal Water Research Project / Biogeochemistry Department; S.Y. Litvin, Stanford University / Hopkins Marine Station. Coastal hypoxia has been increasingly documented in nearshore waters the California Current Large Marine Ecosystem (CCLME). While in other regions of the world, coastal hypoxia has a strong linkage to anthropogenic nutrient inputs, low dissolved oxygen (DO) in California's nearshore has been linked to offshore influences such as upwelling, which can transport hypoxic bottom-waters to the shelf. For Southern California, a heavily populated region which discharges treated municipal effluent as well as urban runoff to the SCB, the extent of coastal hypoxia and the contribution of anthropogenic nutrients versus offshore processes to the problem have not been investigated. In this study, we utilize a 30 year time series of coastal transects (< 15km from shore) collected during quarterly surveys by the four major sanitation districts to investigate spatial patterns and trends in DO concentration. Significant negative trends in DO occurred in all agencies from 1998 to present, though data for several transects dating back to 1981 show a significant but mild positive DO trend prior to 1998. Comparisons with profiles from the CalCOFI time series (inshore of 200 km) showed similar regional trends, with little trend before 1998 and strong negative trends after 1998. While DO concentrations within the nearshore transects has a 3 fold larger negative trend than CalCOFI data, suggesting that anthropogenic inputs may be causing some localized declines in DO, the large regional scale of the declines suggest that negative trends in coastal oxygen are due to changing source waters, brought into the shallow nearshore by upwelling.

**67 Changes over the Past Four Decades in Fishability and Ecosystem Health in the Southern California Coastal Ocean** K. Setty, Southern California Coastal Water Research Project (SCCWRP). Two primary goals of the Clean Water Act were to make the nation's surface waters "fishable" and restore the integrity of aquatic ecosystems. In southern California, many efforts have been made across different sectors to achieve these goals. The Southern California Coastal Water Research Project's Commission's Technical Advisory Group recently undertook a collaborative effort to analyze historical data trends and determine whether conditions in coastal marine waters have improved as a result. Levels of bioaccumulative pollutants like DDT and PCBs have declined dramatically in fish populations. Still, fish consumption advisories remain for much of the coastline, often due to widespread moderate mercury contamination. The incidence of fish disease has also been greatly reduced, and fish and invertebrate community integrity has improved to a fair degree. Birds of prey like the brown pelican are on the rebound, though their recovery has been slowed by legacy contamination. Kelp forests and rocky reef habitat show some positive effects of improved water quality management, but are also impacted by other factors like natural climate cycles, coastal development, and fishing pressure. Future challenges facing regulators and environmental managers include the greater pollution impacts in enclosed coastal embayments and novel water pollution issues like ocean acidification, global climate change, marine debris, and the subtle toxicological effects of emerging contaminants.

**68 Forty Years of Progress: Recreational Water Quality Management in California** J.F. Griffith, Southern California Coastal Water Research Project / Department of Microbiology. California recreational water quality management has evolved steadily over the past decades. With the highest rates of beach visitation in the nation, California's coastal waters are recognized as a valuable economic resource and aesthetic benefit to all. In the past decade multiple examples of regional stakeholder collaboration have led to steady improvement in beach water quality, indicating the common concern for successful ambient water quality management. There are multiple examples of collaborative efforts that have led to water quality improvement, including dry and wet weather diversion of stormwater and urban runoff for treatment and use of advanced modeling and BMP-based approaches for water quality management. California has also led scientific advancement in the area of technology development and implementation including



development and implementation of novel microbial source tracking and rapid beach water quality measurements methods. This presentation will focus on trends in regional water quality and technological advancements.

**69 Long-Term Trends in Water Quality in San Francisco Bay** J.A. Davis, J. Ross, San Francisco Estuary Institute; M. Kellogg, San Francisco Public Utilities Commission; A. Cohen, Center for Research on Aquatic Bioinvasions. A new State of the Bay Report for San Francisco Bay summarizes progress in attaining management goals relating to habitat, water supply and quality, living resources, ecological processes, and stewardship. A water quality evaluation is a component of the Report that assesses whether the Bay is safe for aquatic life, whether Bay fish are safe to eat, and whether the Bay is safe for swimming. Many monitored pollutants are considered to pose very low risk to Bay aquatic life, but a few (especially methylmercury, exotic species, the toxicity of sediments, and trash) pose substantial threats. Several other pollutants appear to pose risks to Bay aquatic life, but definitive regulatory goals for the Bay have not yet been developed. A few prominent examples include selenium, polynuclear aromatic hydrocarbons, and perfluorooctanesulfonate (PFOS). Fish from the Bay are not entirely safe to eat, due mainly to polychlorinated biphenyls (PCBs), methylmercury, and dioxins. Most Bay beaches are safe for swimming in the summer, but bacterial contamination is a concern at a few beaches in the summer, and at most beaches in wet weather. Overall, thanks to the considerable investment that has been made in wastewater treatment infrastructure and the diligent efforts of water quality managers, the Bay is much safer for aquatic life and for people to fish and swim in than it was in the 1960s. Substantial control efforts that began in the 1970s as a result of the Clean Water Act solved most of the obvious problems of the 1960s and set the Bay on a course for gradual recovery for many pollutants. However, challenges, information gaps, and uncertainties remain to respond to legacy contaminants, such as mercury and PCBs, emerging contaminants, nutrients, and other threats to water quality. Complete and timely resolution of remaining and emerging water quality challenges will require significant investments of resources to replace and improve our aging water quality infrastructure.

**70 Meta-Analysis of Selected Puget Sound Initiative Sites: Remediation Progress and Possible Geographic Influences** R. Combs; R. Combs, Western Washington University; A. Spanjer, Western Washington University / Huxley College of the Environment; J. Barnes, D. Cheyette, K. Woolsey, R. Green, Western Washington University; R.M. Sofield, Western Washington University / Huxley College of Environment. This study is a secondary analysis of sediment data comparing different cleanup project successes across the Puget Sound using data from the early 1980s to the present. The analysis focuses on six bays within the Puget Sound identified as part of the Puget Sound Initiative (PSI). It compares current levels of sediment contamination and expected exposure to biota with current state sediment standards and other accepted guidelines for each bay using Apparent Effects Threshold, PEL/TEL, and ERM/ERL values. Measured sediment and tissue concentrations of arsenic, mercury, PAHs, and total PCBs in each bay were retrieved from the Washington State Environmental Information Monitoring System (myEIM) and compared to the sediment standards and guidelines. Percent contamination above the sediment guidelines and standards by surface weighted average concentration area per bay and per chemical, and for percent contamination above the values by year per bay and chemical were calculated. Correlations were also calculated for sediment contamination with a selected range of environmental parameters shown in the literature to influence contamination levels in sediments, such as average sediment loading, freshwater discharge runoff rate and percent land use categories. Of the selected chemicals analyzed, mercury, PCBs, and arsenic had significant correlations with these environmental parameters. Animal tissue data taken synoptically with sediment data was also compared to these same environmental parameters. Future work will include correlating geographic and anthropogenic factors that may influence sediment concentrations and analyzing the progress and stage of remediation each bay is currently engaged.

**71 Changes in the Northeast U.S. as a Result of the Clean Water Act: The Chesapeake Bay Watershed Experience** C.W. Abdalla, Pennsylvania State University / Department of Agricultural Economics, Sociology and Education. The Chesapeake Bay is North America's largest and most biologically diverse estuary and is an important commercial and recreational resource. The Bay is estimated to contribute an annual economic

benefit estimated at \$33 to \$60 billion (2010 dollars). Seventeen million people currently live with the Bay's 64,000 square mile watershed in the District of Columbia or within portions of Delaware, Maryland, New York, Pennsylvania, Virginia and West Virginia. Most of the Bay's waters are listed as impaired because of excess nutrients (nitrogen and phosphorus) and sediment. These pollutants have disrupted the Bay's ecosystem, creating conditions that impact the survival of aquatic life. Man's activities (e.g. land development, agricultural operations, urban and suburban runoff, wastewater discharges, and air pollution) have degraded the Bay. Important phases in public policies and programs implemented under the auspices of the federal Clean Water Act to improve quality of the Chesapeake Bay include: 1) a partnership – the Chesapeake Bay Program – created in 1983 among the EPA, Maryland, Pennsylvania, and Virginia, and the District of Columbia to address the extent, complexity, and sources of pollutants entering the Bay; 2) In 1987, the partners committed to reduce controllable nutrients loadings by 40 percent by 2000; 3) In 2000, the partnership signed an agreement that provided an alternative to developing a Bay-wide Total Maximum Daily Load (TMDL) based on the expectation that actions would be taken to meet water quality standards within the Bay by 2010. 4) In 2007, EPA concluded that despite extensive efforts by federal, multi-state, state/local governments, non-governmental organizations, and other stakeholders insufficient progress had been made toward meeting the water quality goals for the Chesapeake Bay and its tidal waters. As a result, EPA issued a Bay-wide TMDL – the most complex ever approved by EPA – to achieve reductions in nutrient and sediment pollution throughout the Bay watershed. This presentation will review the major phases in federal, multi-state and state policies and programs under the Clean Water Act's authority from the 1980s to the Chesapeake Bay-wide TMDL that is currently being implemented. Observations will be made about each phase's goals, approaches, outcomes, limitations and lessons learned.

**72 Ambient water quality in the Southeastern US: Trends and advancements in water quality management** R. Noble, D. Blackwood, UNC Chapel Hill / Institute of Marine Sciences. In the past several decades, the coastal and estuarine shorelines of the Southeastern USA have undergone dramatic development, resulting in increased use and appreciation of the beautiful beaches, but with concomitant increases in the delivery of microbial contaminants to receiving waters due to stormwater, increased impervious surface coverage, and increased sewage inputs, to name a few. For the past several years, states such as North Carolina have risen in their importance and rate of visitation from tourists, and the recreational activities possible in this region have expanded to include kayaking, canoeing, parasailing, ecotourism, kitesurfing and surfing. In response to the growth and development along the coastlines of Virginia, North Carolina, South Carolina, Georgia, and Florida, increased attention has turned to stormwater research, stormwater controls, and stormwater mitigation, as storms are major forces contributing pulses of microbial contaminants to systems. Storms are highly variable, with ranging levels of intensity and duration corresponding to seasonal change and vegetation. Extreme storm events play an important role in the contamination of surface waters of the region, with hurricanes and Nor'easters sometimes causing contamination that persists over the duration of months. Furthermore, common BMPs are of limited use due to the shallow nature of the water table, and flat landscape. We will present case studies that demonstrate some of the major issues facing the Southeastern states in preserving and restoring surface water quality. In addition, we will discuss trends in water quality over the past decades, including an examination of major trends in water quality in North Carolina using multiple linear regression techniques. When considering long term water quality management in the Southeastern states, a common theme is watershed based management, because watersheds are vast due to the topographic nature of the flat coastal plain. Therefore, a common thread for this presentation will be the importance of the linkage of data from tidal streams through estuaries to the barrier island beaches. The success of water quality management in this region is dependent, finally, upon stakeholders of all perspectives working together to solve problems. Highlights in successful water quality management over the past decades, along with observation of trends and consideration for the future will be presented.

**73 Birds as a Tool in the Great Lakes AOC Delisting Process** C.M. Custer, U.S. Geological Survey / Upper Midwest Environmental Sciences Center, U.S. Geological Survey / Upper Midwest Environ. Sciences Center; T.W. Custer, U.S. Geological Survey / Upper Midwest Environmental

Sciences Center, U.S. Geological Survey; P.M. Dummer, U.S. Geological Survey. Two wildlife-related Beneficial Use Impairments (BUIs) are present at many of the Great Lakes' Areas of Concern (AOCs): Bird or Animal Deformities or Reproduction Problems and Degradation of Fish and Wildlife Populations. Tree swallows (*Tachycineta bicolor*) are an ideal species to address these two BUIs because they can be attracted to specific AOCs, even to highly urbanized and industrial environments, in large enough numbers to collect statistically valid reproductive and contaminant exposure data. Because tree swallows are aquatic insectivores, contaminant concentrations in their tissues reflect what is present and bioavailable in AOC sediments. Nest boxes can be placed so that their feeding range represents sediment contamination at the AOC and not other aquatic habitats in the general vicinity. Because of the wide-spread use of tree swallow for contaminant assessment studies, there are benchmark contaminant-effect threshold values now available for various contaminant classes. This Great Lakes Restoration Initiative funded project has now collected three years of data at 20 AOCs encompassing 40 unique study sites, as well as, data from 6 nearby reference areas. Data have been collected on both organic and inorganic contaminants including legacy contaminants of concern (e.g., polychlorinated biphenyls [PCBs], dioxins and furans, pesticides, and mercury), as well as many new and emerging contaminants such as polybrominated diphenyl ethers (PDBEs) and perfluorinated compounds (PFCs). Data have also been collected on reproductive, physiological, and genetic endpoints at each of these sites, and will be used in conjunction with contaminant concentrations to ascertain whether there is an association between chemical exposure and these effect endpoints. These data are providing a valuable tool for both State and Federal agencies to assess the status of these two wildlife-related BUIs and whether they can be removed.

#### **74 Demonstrating the restoration of Great Lakes AOC Beneficial Uses: Ashtabula River Case Study**

**A.P. Mucha**, U.S. Environmental Protection Agency / Great Lakes National Program Office; A. Klei, P. Anderson, Ohio EPA; M.A. Mills, U.S. EPA, U.S. EPA / National Risk Management Research Laboratory, ORD, US EPA / Remediation and Pollution Control Division, NRMRL, U.S. EPA / Office of Research & Development; S. Cieniawski, U.S. Environmental Protection Agency / Great Lakes National Program Office. The Great Lakes designated 43 Areas of Concern (AOCs) are defined by the number and extent of Beneficial Use Impairments (BUIs) present. The 14 potential BUIs span a wide range of impacts and endpoints and include restrictions on dredging, restriction on fish and wildlife consumption, and degradation of benthos. To restore AOCs, individual BUIs must be removed and requires building a scientific case demonstrating improvement. The Ashtabula River AOC is a priority site where all key management actions must be completed by 2012. At the Ashtabula River AOC, there are currently 6 BUIs, including degradation of benthos, fish tumors and deformities, and restrictions on fish and wildlife consumption. To effectively document the function of the ecosystem and to assess the status of the beneficial use impairments requires extensive data and documentation. Over 500,000 yds<sup>3</sup> of contaminated sediment was dredged from the river along with in water and riparian habitat restoration under the Great Lake Legacy Act Program in 2007. Additional and more significant habitat restoration was done by Ohio EPA using GLRI funding. In 2011, a multi-Agency sampling effort took place on the Ashtabula AOC to address multiple regulatory and AOC goals. The data that resulted from this effort were critical in defining actions needed at the AOC as well as help define the status of restoration at the AOC. The plan was a multiple Agency effort involving EPA, USFWS, NOAA, and the Ohio state agencies. The sampling and data collection included such measures as surface weighted average concentration (SWAC) established with surface sediment concentrations, biota contaminant concentrations (i.e. fish, macrobenthos), etc. These data have been collected and compared to historic data collected historically and immediately prior to remediation and restoration. Trends indicate that there has been recovery within the AOC following the management actions to date. These data will be presented and discussed as well as additional management actions that remain.

#### **75 Expanded Mussel Watch AOC Assessment as part of Great Lakes Restoration Initiative**

**K. Kimbrough**, W. Johnson, NOAA/National Centers for Coastal Ocean Science / NCCOS Mussel Watch Program; T. Nalepa, NOAA/GLERL / GLERL; J. Hyland, NOAA/National Centers for Coastal Ocean Science / Center for Coastal Environmental Health and Biomolecular Research; M. Fulton, NOAA/National Centers for Coastal

Ocean Science / Marine Ecotoxicology Division; A. Jacob, Consolidated Safety Services. NOAA's Mussel Watch, a nationally recognized contaminant monitoring program, has been monitoring chemical contaminants in the Great Lakes since 1992. Two decades of monitoring has documented the continuing presence of legacy contaminants and heavy metals at many locations within the Great Lakes. Recently, through coordination with Great Lakes Restoration Initiative, Mussel Watch has expanded its monitoring efforts to include all U.S. Great Lakes Areas of Concern (AOC), with the objective of providing data and information in support of decision making for removal of Beneficial Use Impairment targets and delisting of AOCs. In addition to routine monitoring of more than 150 contaminants in bivalve tissues, Mussel Watch will provide data on sediment contamination, benthic infaunal composition, sediment toxicity and cellular biomarkers of exposure and effects. Data from AOCs can be meaningfully interpreted by comparisons to long-term historic Mussel Watch sites, which can provide important baseline data as well as serve as reference sites. Enhanced Mussel Watch efforts can thus aid resource managers and policy makers in making informed management decisions, and contribute to the overall restoration and protection of the Great Lakes.

#### **76 Fish Population Modeling Approaches for Assessing Direct Effects and Recovery Following Mitigation of a Pulp Mill Effluent in Jackfish Bay**

**D.H. Miller**, US EPA / Office of Research and Development, National Health and Environmental Effects Research Laboratory, Mid-Continent Ecology Division; J.E. Tietge, US EPA / Office of Research and Development, National Health and Environmental Effects Research Laboratory, Mid-Continent Ecology Division; M.E. McMaster, Environment Canada / National Water Research Institute; K.R. Munkittrick, University of New Brunswick / Canadian Rivers Institute, University of New Brunswick / Department of Biology; X. Xia, D.A. Griesmer, Computer Sciences Corporation; G.T. Ankley, US EPA / Office of Research and Development, National Health and Environmental Effects Research Laboratory, Mid-Continent Ecology Division. We present an approach to link chemically induced alterations in molecular and biochemical endpoints to adverse outcomes in whole organisms and populations. A predictive population model was developed to translate changes in fecundity measures of white sucker (*Catostomus commersoni*) collected at Jackfish Bay, Lake Superior to alterations in population growth. Individual-level responses of fish exposed to pulp mill effluent were used to demonstrate the model's capability to project alterations in population status, both in terms of ongoing impact and subsequent recovery after stressor mitigation associated with process changes at the mill. Long term monitoring data from the Jackfish Bay site includes biochemical endpoints, such as steroid measurements, relevant to an adverse outcome pathway (AOP) resulting in decreased fecundity. In combination with population modeling, the AOP framework can be used as an organizing principle by which mechanistic data (e.g. circulating steroid concentrations) can be effectively translated into endpoints meaningful to ecological risk (e.g. population status). Extrapolation of the population modeling construct demonstrated at the Jackfish Bay site can be made to characterize white sucker population trends at other sites that are less data rich. This abstract does not necessarily reflect U.S. EPA Policy.

#### **77 Metabolomic Approaches for Characterizing Impaired Aquatic Ecosystems**

**T. Collette**, U.S. EPA / National Exposure Research Laboratory; G.T. Ankley, US EPA / Office of Research and Development, National Health and Environmental Effects Research Laboratory, Mid-Continent Ecology Division; E. Durhan, US EPA / Mid-Continent Ecology Division / Research Chemist; K. Jensen, M. Kahl, U.S. EPA / National Health and Environmental Effects Research Laboratory; K. Lee, U.S. Geological Survey; E. Makynen, US EPA / Mid-Continent Ecology Division; D. Skelton, U.S. EPA; Q. Teng, US EPA / National Exposure Research Laboratory; A. Trowbridge, U.S. Fish and Wildlife Service; D.L. Villeneuve, U.S. EPA / National Health and Environmental Effects Research Laboratory, U.S. EPA; D.R. Ekman, US EPA / National Exposure Research Laboratory. Metabolomics is becoming a well-established tool for studying how organisms, such as fish, respond to various stressors. For example, the literature is rich with laboratory studies involving analysis of samples from organisms exposed to individual chemical toxicants. These studies have demonstrated the ability to rapidly screen and prioritize chemicals for adverse effects, and to determine mode-of-action-specific exposure biomarkers. In addition, there has been promising laboratory work with metabolomics for characterizing an organism's compensation and recovery from toxic effects, and for defining



the complex temporal nature of responses to stressors. Our group has recently leveraged information gained from these laboratory studies in order to use metabolomics for biological-based exposure monitoring in Great Lakes Areas of Concern (AOCs), using field-deployed caged fish. One promising outcome of this work has been recognition of the potential to correlate adverse outcomes (as measured by metabolomics) with individual stressors that occur in the AOCs. For example, with this approach, information from metabolomics may contribute to the weight of evidence that a particular chemical contaminant (or class of contaminants) is causing a particular Beneficial Use Impairment (BUI) within an AOC. Information of this type could be very valuable for designing effective remediation strategies. In addition, metabolomics has emerged as an ideal candidate tool for characterizing recovery trends in fish and other organisms, suggesting its use in long term biological-based monitoring studies over the course of restoration activities. As such, metabolomics may provide critical information in ongoing efforts to eliminate BUIs and to delist AOCs. Results from preliminary studies that illustrate the potential for these applications will be presented.

#### **78 Pathway-based Monitoring of Biological Effects at Great Lakes sites**

N. Vinas, Mississippi State University; D.L. Villeneuve, U.S. EPA / National Health and Environmental Effects Research Laboratory; L. Escalon, US Army Engineer Research and Development; T. Habib, BTS; G.T. Ankley, US EPA / Office of Research and Development, National Health and Environmental Effects Research Laboratory, Mid-Continent Ecology Division; E. Durhan, US EPA / Mid-Continent Ecology Division; M. Kahl, K. Jensen, U.S. EPA / National Health and Environmental Effects Research Laboratory; J. Cavallin, U.S. EPA, ORISE / National Health and Environmental Effects Research Laboratory; E.J. Perkins, US Army Engineering Research & Development / Environmental Laboratory, US Army ERDC / Environmental Processes & Effect Division, US Army Engineering Research & Development / Environmental Genomics and Genetics Team. The Great Lakes region suffers from degradation of water and environmental quality due to release of chemicals of emerging concern (CEC) that may threaten near shore health. Critical issues remain in delisting AOC including determining sources of chemicals causing fish health impacts (sediment, sewage overflow, effluent discharges, or tributary waters), relating health impacts to chemical exposure or specific source points, and identifying causes of adverse health effects such as tumors and reproductive effects. Causal information would enable decision makers to identify more appropriate remedial actions thereby facilitating delisting of AOC. Fish placed in floating cages at different locations within AOC can be used to rapidly monitor the impacts of chemicals on fish health by monitoring chemicals in water, fish, and resulting impacts to fish health in terms of reproductive, physiological and genetic measures. Specific changes found in fish organs such as gonads and liver can be associated with specific effects. For example impacts on egg protein and hormone production related genes are associated with classes of chemicals causing reproductive impacts. Similarly changes in specific genes can be associated with exposure to polycyclic aromatic hydrocarbons and other chemicals. We are using molecular changes in liver and gonad tissues to identify the health impacts, if any, caused by several high-priority AOC. We will also use gene changes in liver and gonads to identify possible chemicals of concern based on gene changes associated with specific chemical exposures in the laboratory. We expect that this work will identify any potential health effects in fish leading to more accurate assessment of contaminant effects on near shore health. We also expect that this would provide more accurate monitoring of effects during restoration and other efforts in the Great Lakes Restoration Initiative program.

#### **79 Riparian indicators of PCB exposure in Manistique River and Harbor AOC**

D. Walters, USGS / Fort Collins Science Center; M.A. Mills, U.S. EPA / National Risk Management Research Laboratory, ORD, US EPA / Remediation and Pollution Control Division, NRMRL, U.S. EPA / Office of Research & Development; A.P. Mucha, U.S. Environmental Protection Agency / Great Lakes National Program Office; S. Cieniawski, U.S. Environmental Protection Agency / Great Lakes National Program Office. The Manistique Area of Concern (AOC) in northern Lake Michigan is contaminated with polychlorinated biphenyls (PCBs). This AOC includes ~2.8 km of the Manistique River and Harbor as well as backwater habitats that are part of a system of docks constructed during an earlier lumbering era. These backwaters are connected to historic PCB sources and remain prone to high, patchy sediment PCB concentrations. We initiated a 2011 study designed to 1) characterize aquatic habitats (backwaters, river, harbor, and

Lake Michigan) using biogeochemical attributes; 2) quantify PCB exposure in riparian predators relative to contaminated sediments; and 3) develop indicators and tools to assess the effectiveness of future remedies. We collected water quality data, sediments, and riparian spiders (Araneida and Tetragnathidae) at 10 sites encompassing backwater, river and harbor sites, an upstream reference site, and a beach/lake site that is subjected to episodic and large pulses of woodchips and sawdust from Manistique River. Backwaters had distinctive biogeochemistry due to a combination of factors related to groundwater inputs (e.g., higher conductivity) and ecosystem processes (e.g., lower DO). Sediment concentrations in the AOC varied from 135 – 10200 ng/g ΣPCB (dry weight) and were typically higher at backwater and harbor sites. Spider concentrations varied from 47 – 370 ng/g ΣPCB (wet weight) and were significantly, positively correlated with sediment ΣPCB<sub>TOC</sub> concentrations ( $r^2 = 0.48$ ;  $p = 0.002$ ). Sediment PCBs within the AOC were dominated by low-chlorinated congeners and were strongly dissimilar from lake and reference sites. We conclude that (1) distinctive chemistry and organic matter dynamics in backwaters could influence PCBs cycling and bioaccumulation within these habitats as well as PCB fate and transport within the AOC; 2) spiders are good indicators for assessing riparian PCB exposure and for assessing the effectiveness of future remedies; and 3) patterns of congener distributions can provide critical information regarding source control and remedy effectiveness.

#### **80 Mink jaw lesions, a sensitive indicator criteria for BUI delisting**

M.J. Zwiernik, Michigan State University, Wildlife Toxicology Laboratory / Department of Animal Science, College of Veterinary Medicine; W.R. Folland, Michigan State University, Wildlife Toxicology Laboratory / Department of Animal Science, College of Veterinary Medicine; J. Moore, Michigan State University, Wildlife Toxicology Laboratory / Dept. of Animal Science; S.J. Bursian, Michigan State University / Department of Animal Science. The ultimate goal for any Area of Concern (AOC) is the elimination of Biological Use Impairments (BUIs) and subsequent site delisting. One of major obstacle to AOC delisting is that BUIs are often noted in multiple ecological compartments with assessment endpoints that are not clearly linked to measureable goals. Here we present a newly identified sensitive biomarker in a recognized sentinel species to identify contaminant exposure and establish a measurable criteria for ecologically relevant BUI listing and delisting for AOCs containing polychlorinated biphenyls, dioxins, and other dioxin-like compounds (DLCs). The American mink is an ideal sentinel receptor species for assessing the ecological risk of AOCs containing DLCs, because mink are exquisitely sensitive to this class of compounds and have great exposure potential as territorial, year round resident apex predators. We have identified jaw lesions as a sensitive biomarker that responds over a wide range of DLC exposures in wild and controlled study mink exposed to DLCs. The biomarker is easily quantifiable by gross examination in severe cases, resulting in visible lesions and microscopically for animals exposed to concentrations 2- to 10-fold lesser than those known to adversely affect reproduction and health. The direct relationship between dose and response severity, anchored on the most severe end with an ecological relevant response, adds utility to the response as a biomarker allowing an estimation of risk distribution based on the severity of the biomarker as seen among individuals or populations. The majority of the response curve is not associated with an adverse impact on individuals thus its presence is not selected against within the population. This is important because growth and reproductive parameters are often key assessment endpoints associated with ecological BUIs criteria, however both are virtually impossible to measure in the field. In addition to the biomarker distribution, abundance, demographics, and morphological measures can also be used to assess population structure and status, which can provide additional support to BUI delisting criteria. Finally, mink tissues may be easily and inexpensively obtained as byproducts of the trapping industry thus acquiring individuals often require minimal effort while providing public education and involvement.

#### **81 Tidal induce transport of mercury in salt-marsh sediments**

L. Canario, IPMA I.P. / Aquatic Environment and Biodiversity; M. Caetano, IPMA I.P.; L. Poissant, Environment Canada; C. Vale, IPMA I.P.; N.J. O'Driscoll, Acadia University, Acadia University / Department of Earth & Environmental Sciences. Intertidal environments are characterized by complex subsurface hydrologies regulated by topography, sedimentology, groundwater discharge, as well as tidal pressure and wave action. In these environments vertical flow at ebb tide increase hydrostatic pressure at depth and forces the release of pore water to the surface. In order to assess how

this force influences mercury fate in intertidal sediments and the associated biogeochemical processes, several inundation experiments were performed in vegetated and non-vegetated intertidal sediments in three sites of the Tagus Estuary (Portugal). Sediments and overlying water were collected at a high temporal resolution after inundation at each sampling site. Dissolved gaseous mercury (DGM) and water-to-air volatilization was measured using a floating dynamic flux chamber. In the laboratory, water (pore water – PW – and overlying water – D) samples were analysed for dissolved mercury species ( $\text{Hg}_\text{D}$  and  $\text{Hg}_\text{PW}$ ), methyl mercury ( $\text{MeHg}$ ), dissolved organic carbon and suspended particulate matter (SPM) content. Salinity, dissolved oxygen and pH were measured in situ. Solid sediments were analysed for Hg, MeHg and organic carbon content. In vegetated sediments Hg and MeHg content were also determined in the root biomass. Concentrations of dissolved mercury species in flooded water increased up to 10 times during the first 5 minutes of tidal flooding in vegetated and non-vegetated sediments. Concurrently MeHg concentrations decreased in solids and in pore waters. A strong correlation ( $R = 0.99$ ;  $p < 0.01$ ) was found between mercury volatilization from water and DGM concentrations indicating the escape of recently formed  $\text{Hg}^0$  to the atmosphere. During the inundation of vegetated sediments MeHg concentrations in roots decreased as much as 31% in the first 8 minutes after the inundation. This suggests that MeHg is far mobile that would be expected if the sequestration processes in roots was dominated by affinity to cysteine-rich peptides. The short-time variations registered in this work indicates a shift in the mercury equilibrium between pore waters, solid sediments and root surfaces. As water floods salt marsh sediments, MeHg, escapes from the sediments being potentially available to biota living those environments. The results obtained in this study clearly points to the importance of tides in Hg and MeHg export from sediments, DGM formation and Hg volatilization in shallow meso- and macrotidal estuaries.

**82 Determining rate constants for photochemical reduction of mercury in Arctic snow – Effects of site specific factors and irradiation intensity** E. Mann, Memorial University of Newfoundland / Environmental Science, University of Toronto Scarborough / Chemistry; N. O'Driscoll, M. Mallory, Acadia University; A. Qureshi, Harvard University; T. Avery, Acadia University; J. Dalziel, R. Tordon, Environment Canada; S. Ziegler, Memorial University of Newfoundland. The arctic is a region where mercury (Hg) contamination is a problem. Snow is an important scavenger for atmospheric mercury, and once present in the snowpack, Hg may be transported to aquatic food webs. Photochemical oxidation and reduction reactions largely control mercury retention in snow; however, the rates of these reactions are uncertain. To accurately model mercury behavior in Arctic regions and to better understand exposures and risk, reliable Hg reduction rate constants are required. This work presents photoreduction rate constants for mercury in solid snow with varying radiation intensity and snow chemistry. Snow was sampled in the high Arctic from 3 sites near Resolute, NU in March 2012 using 2L Teflon containers and clean techniques. Triplicate samples were exposed to a range of environmentally realistic intensities of ultra-violet radiation ( $1.26 - 5.78 \text{ W/m}^2$  for  $\lambda = 280$  to  $400 \text{ nm}$ ) using a quartz beaker inside a Luzchem photoreactor maintained at  $-10^\circ\text{C}$ . Elemental mercury produced in snow was quantified using continuous analysis by gold amalgamation AFS (Tekran model 2537A). Pseudo-first order reaction rate constants were found to range between  $0.5 \pm 0.07 \text{ h}^{-1}$  and  $0.1 \pm 0.03$  for triplicate analyses. These rate constants were then compared over the studied irradiation intensities, and the range of snow chloride and DOC concentrations observed, to generate empirical relationships for pseudo-first order reaction rate constants. Mercury reduction rate constants were linearly related to UV intensity ( $R^2 > 0.9$ ,  $p < 0.05$ ). This study is the first to generate controlled pseudo-first order mercury reduction rate constants in snow which will be useful for modeling mercury retention in the Arctic.

**83 Total and monomethyl mercury in fog water and spiders from the central California coast** P. Weiss-Penzias, University of California Santa Cruz / Microbiology and Environmental Toxicology; C. Ortiz, University of California, Santa Cruz. Total mercury ( $\text{HgT}$ ) and monomethyl mercury (MMHg) concentrations in fog collected from 4 locations in and around Monterey Bay, California during June-August of 2011 were  $10.7 \pm 6.8$  and  $3.4 \pm 3.8 \text{ ng L}^{-1}$  respectively. In contrast, mean  $\text{HgT}$  and MMHg concentrations in rain water from March-June, 2011 were  $1.8 \pm 0.9$  and  $0.1 \pm 0.04 \text{ ng L}^{-1}$  respectively. Using estimates of fog water deposition from 6 sites in the region using a standard fog water collector (SFC), depositions of  $\text{HgT}$  and MMHg via fog were found to range from 42-4600 and 14-1500

$\text{ng m}^{-2} \text{ yr}^{-1}$ , which accounted for 7-42% of  $\text{HgT}$  and 61-99% of MMHg in total atmospheric deposition (fog, rain, and dry deposition), estimated for the coastal area. These initial measurements suggest that fog precipitation may constitute an important but previously overlooked input of MMHg to coastal environments. Preliminary comparisons of these data with associated chemical, meteorological and oceanic data suggest that biotically formed MMHg from coastal upwelling may contribute to the MMHg in fog water. Wolf spiders collected at locations the Monterey Bay were also analyzed for  $\text{HgT}$  and MMHg with the goal of determining if Hg could be accumulating in these species due to fog deposition. We found mean concentrations of  $\text{HgT}$  and MMHg of 360 and 270  $\text{ng g}^{-1}$ , respectively. Comparing these results with previous studies on Hg in orb-weaving spiders and mosquitos, suggests that spiders in the Monterey Bay area have enhanced concentrations, similar to those found in insects near Hg contaminated sites.

**84 Migration and mercury in fish-eating birds** R.A. Lavoie, Queen's University / Biology; K. Kyser, Queen's University / Geological Sciences & Geological Engineering; L. Campbell, Saint Mary's University / Biology. Birds can transport contaminants between ecosystems during their annual cycle (e.g., through feather molt) and this leads to unexplained variations in mercury (Hg) concentrations within and among bird species. The objective of this study was to test whether migration explains variations in Hg concentrations observed in migratory fish-eating birds. Two migratory piscivorous bird species, the double-crested cormorant (*Phalacrocorax auritus*; short migrant) and the Caspian tern (*Hydroprogne caspia*; long migrant) were caught during the breeding season in Lake Ontario (Laurentian Great Lakes) and feathers grown during the winter and summer were collected to analyze for total Hg and stable carbon ( $\delta^{13}\text{C}$ ), nitrogen ( $\delta^{15}\text{N}$ ), hydrogen ( $\delta\text{D}$ ) and sulfur ( $\delta^{34}\text{S}$ ) isotopes to estimate wintering habitats and migration. Light-level geolocators were installed on birds for a full annual cycle to validate stable isotope estimations of wintering sites. Geolocators were also used to measure the importance of migration sites on Hg exposure. Results indicate that a proportion of individuals had higher total Hg concentrations than the adverse effect threshold for adult piscivorous birds. Mercury and isotopic values were highly variable for winter feathers which suggest high intra- and inter-specific migration patterns and Hg exposure. On the other hand, summer feathers showed little intra- and inter-specific variation consistent with similar breeding habitat and diet. Specific relationships between Hg, stable isotopes and geolocators will be presented. This research project allowed a better understanding of the effect of migration on Hg concentration and the importance of biovector transport of Hg through birds.

**85 Design of a mechanistic model for mercury bioaccumulation in fish: a bioenergetic-pharmacokinetic approach** C.A. Ng, ETH, ETH Zurich / Inst. for Chemical and Bioengineering, S&U Group, ETH Zurich / Institute for Chemical and Bioengineering; K. Hungerbuehler, Swiss Federal Institute of Technology (ETH Zurich) / Institute for Chemical and Bioengineering. As we approach the first internationally binding treaty on mercury, this pervasive environmental pollutant remains one of the primary chemicals of toxic concern in aquatic ecosystems. Given its long history of use and known harmful effects, research efforts continue to pursue a better understanding of its fate, global distribution and eventual exposure in organisms. Consumption of contaminated fish is a leading exposure pathway for human populations, yet the mechanisms determining mercury fate in aquatic ecosystems are still poorly described. Importantly, there is a need for a more mechanistic understanding of its bioaccumulation in aquatic food webs. Here, we develop a bioenergetics-based pharmacokinetic model for mercury bioaccumulation in fish. Our model has two key features; first, it explicitly considers the uptake routes via gill and gut surfaces in fish for different mercury species, including both passive diffusion (for neutral organic mercury) and facilitated transport (for charged species). Second, the model uses a pharmacokinetic framework to predict the transport to and accumulation of mercury species in different fish tissues. This framework is informed by recent empirical work tracking tissue distribution in both aquatic ecosystems and from the more substantial mammalian pharmaceutical literature, and may help provide some insights into the accumulation of proteinophilic (protein-associated) chemicals in organisms. The model features are aimed at enabling application to multiple sites and multiple species based on the parameterization of the bioenergetic component, and thus removes one of the main limitations of empirical site-specific models. It may also aid in developing risk assessments for mercury toxicity that benefit from knowledge regarding tissue-specific and mercury-species-specific body burdens.

**86 Non-steady state mercury bioaccumulation and its drivers in predatory fish** R. Abma, University of Windsor / Great Lakes Institute for Environmental Research; D. Haffner, University of Windsor / GLIER; G. Paterson, University of Windsor / Great Lakes Institute for Environmental Research. Mercury toxicity has been a relevant, global issue since the 1950s, when fish consumption was discovered to be the primary route of human exposure to the toxin. Mercury was responsible for the closure of many fisheries in the Great Lakes region in the 1970s, and since then mercury levels have been monitored and government programs have been put in place to encourage cleanup and prevention of mercury pollution. Despite these efforts, however, fish tissue levels of mercury remain a concern. Understanding the bioaccumulation of mercury in fish tissues has been an important focus in the Great Lakes region. Bioaccumulation models have been used to predict the dose of mercury that enters Great Lakes food chains, the level of mercury in top predators, and mercury exposure to humans through consumption of fish. Current mercury bioaccumulation models assume steady state dynamics, but accumulation patterns rarely follow this trend. This study investigated mercury accumulation in lake trout over time, and results revealed exponential increase of mercury concentration in fish tissues with increasing age. Thus, mercury accumulation reflects non-steady state dynamics. Potential causes for this accumulation pattern were also explored. Trout from the three basins of Lake Huron exhibit different overall mercury body burdens and growth rates. Growth dilution were explored as a regulator of non-steady state mercury accumulation both within and among lake trout populations. Growth dilution was shown to regulate mercury accumulation within populations in Lake Huron, with slow growers accumulating mercury at a significantly faster rate than fast growers. These results emphasize the need for non-steady state mercury bioaccumulation models, and promote further study on the nature of mercury bioaccumulation dynamics.

**87 Combined Role of Natural Organic Matter and Base Cations on Hg(II) uptake** S. Chiasson-Gould, University of Ottawa / Biology; J. Blais, Ottawa University; A. Poulain, University of Ottawa / Biology. Despite the alarming trends of declining base cation concentrations in boreal lakes, no studies have attempted to predict the consequences of this decline on the geochemical cycle of mercury, a top priority contaminant worldwide. In this study, we used a whole-cell gram-negative bioreporter to evaluate the direction and magnitude of changes in net accumulation of  $\text{Hg}^{\text{II}}$  by bacteria in response to changing base cation concentrations. We show that regardless of the speciation of  $\text{Hg}^{\text{II}}$  in solution, increasing divalent base cation concentrations decrease net  $\text{Hg}^{\text{II}}$  accumulation by the bioreporter, suggesting a protective effect of these cations. This work suggests that the complexity of the cell wall of gram-negative bacteria must be considered when modeling Hg uptake pathways; we propose that base divalent cations contribute to hamper net  $\text{Hg}^{\text{II}}$  accumulation by decreasing outer membrane permeability and, therefore, the passive diffusion of  $\text{Hg}^{\text{II}}$  species to the periplasmic space. To further test the environmental relevance of this finding with performed a series of experiments investigating Hg uptake in the presence of both base cations and natural organic matter consisting of fulvic and humic acids.

**88 The Impact of Eutrophication on Mercury Cycling in Lake 227 at the Experimental Lakes Area in Northwestern Ontario** J.L. Kirk, Environment Canada / Research Scientist, Environment Canada / Aquatic Contaminants Research Division, Environment Canada; I. Lehnher, University of Waterloo; A. Gleason, Environment Canada; D. Muir, Environment Canada / Aquatic Ecosystem Protection Research Division, Environment Canada / Aquatic Contaminants Research Division; V. St Louis, Environment Canada. Mercury (Hg) is a pollutant of global concern as concentrations of methyl mercury (MeHg), the toxic and bioaccumulative form of Hg, in fish are often present at levels high enough to pose health risks to consumers. Although we are beginning to understand the factors controlling MeHg production in freshwater lakes, the impacts of environmental disturbances, such as eutrophication, on Hg cycling are not known. As part of a larger project examining controls on eutrophication, we are studying the impacts of eutrophication on Hg cycling and MeHg production in the artificially eutrophic Lake 227 at the Experimental Lakes Area in northwestern Ontario. In addition to 40 years of ancillary data, Lake 227 is ideal for this study as it has an anoxic hypolimnion which may be an important zone of microbial MeHg production. To determine sources and losses of inorganic  $\text{Hg}(\text{II})$  and MeHg from the lake, we are using a mass balance approach including: detailed lake profiles to determine the water column pools of inorganic  $\text{Hg}(\text{II})$  and MeHg,  $\text{Hg}(\text{II})$  and MeHg inputs via

precipitation, and losses of Hg from the lake via gaseous elemental  $\text{Hg}(0)$  evasion and MeHg photodemethylation. In addition, rates of water column MeHg production are being determined using state-of-the-art Hg stable isotope experiments. 2010 results suggest that eutrophication has a dramatic impact on the Hg cycle in Lake 227. For example, the zone of elevated water column MeHg migrated throughout the summer following the zone of anoxia, suggesting MeHg is being produced in the anoxic hypolimnion. 2011 results will be presented in the context of phosphorus and nitrogen cycling as well as numerous other water chemistry parameters.

**89 Risk assessment of rare earth metals on ecological receptors at a hypothetical mine development** J.R. Phibbs, University of Saskatchewan / Toxicology Centre, AECOM / M.Sc. Candidate; M. Sanborn, AECOM. The use of ecological risk assessments in support of mining environmental approvals in the last half decade has become more formalized and quantitative in nature. The use of a risk assessment during the planning and approval stage of a proposed mine facilitates quantitative predictions of risk which aids the development of future operational management goals, facilitates long term monitoring requirements/goals and creates greater transparency. The rare earth metals (REM), niobium and tantalum are in high demand for military applications such as superalloys, magnets, and lasers used in propulsion and combustion equipment. Canada has many potential high grade REM resources that are rapidly being evaluated in order to meet the increasing world demand. Viable rare earth metal developments typically have co-occurring radionuclides associated with the deposit. We propose to quantify terrestrial and aquatic ecological risks at a simulated REM mine development with co-occurring radionuclides from a hypothetical mineral deposit based on publicly available geological information. The radionuclides that will be assessed as part of the aquatic and terrestrial ecological risk assessment are the long lived parent and daughter products of the uranium-238, uranium-235, and thorium-232 decay chains. These radionuclides transfer naturally into vegetation, terrestrial receptors and aquatic receptors via food, water and air. In aquatic systems impacted by mine activities the surface water and sediment pathways to aquatic invertebrates, fish, amphibians, and aquatic plants are generally considered to be complete for both REMs and radionuclides. This study aims to identify elements and radionuclides of importance to ecological receptors, and quantify the magnitude of risk using a quantitative risk assessment framework. Preliminary results indicate that potentially significant exposures may be limited to radionuclides and the rare earths with high crustal abundance. Overall, the results of this study will add to our understanding of the potential risks caused by these types of mining developments and highlight the unique challenges of quantifying risks caused by the development of REM ore bodies with co-occurring radionuclides.

**90 Human health risk assessment of the rare earth metals and radionuclides: a simulation study based on a hypothetical Canadian mine** M. Sanborn, AECOM; J. Phibbs, AECOM Canada Ltd.. The rare earth metals (REM) niobium and tantalum are used in nickel-, cobalt- and iron-base superalloys for such applications as jet engine components, rocket subassemblies, and heat-resisting and combustion equipment. The increasing demand for REMs coupled with China's decision to reduce/cease export has resulted in increased interest in exploration and development of REM deposits globally, and particularly in Canada. The development of REM mines can involve additional issues for examination when seeking environmental/regulatory approvals in comparison with base metal mines. The use of environmental risk assessment in support of mining environmental approvals in the last half decade has become more formalized and quantitative in nature. Quantitative predictions of risk can reduce enhance transparency, reduce the potential for disagreements that would otherwise arise from over-reliance on professional judgement, and can aid in the development of risk management/mitigative needs including future operational management goals, and long term monitoring requirements/goals. We present here a human health risk assessment of a simulated REM mine in Canada. The study is based on publicly available information gathered from the Geological Survey of Canada. Exposure assumptions for human receptors are based on values published by Health Canada and the US EPA. Niobium and tantalum regularly co-occur with uranium, thorium and other naturally occurring radioactive elements. While heavy metals are undoubtedly of concern as well, this study is focused on the human health risks associated with REMs and co-occurring radionuclides. Uranium and thorium series radionuclides transfer naturally into vegetation, terrestrial receptors and aquatic receptors



via food, water and air. Human receptors can be exposed to REMs and radionuclides through the ingestion, inhalation, or contact pathways. This simulation study will be used to assess potential risks associated with a typical remote rare earth element mine to the workers and local people involved in traditional land use activities. Results of this study, supported by lessons learned from other active rare earth element mines, indicate that radiological risks can be significant. This study further examines the chemical risks of rare earth elements and provides support for the careful consideration and management of waste streams originating from rare earth element extraction activities.

**91 Sorption of Munitions Constituents by Soil** R. Gonzalez, University of Delaware / Department of Civil and Environmental Engineering; D.M. Di Toro, University of Delaware / Dept. of Civil & Environmental Engineering; H.E. Allen, University of Delaware / Dept. Civil & Environmental Engineering. Many military ranges contain high residual amounts of munitions constituents (MCs) in their soil. These residues may be transported to groundwater thus posing a risk and potentially limiting site use. We have studied adsorption and desorption of MCs on several soils with different properties and have developed new models to describe the partitioning. There is considerable literature on the adsorption of MCs, particularly nitroaromatic compounds, onto soils. These reports focus on whether adsorption is controlled by the organic matter or the clay in soil. Our adsorption model incorporates both organic matter and clay with predictive capability significantly better than a model in which only one of the factors was considered.

**92 Modeling Adsorption and Desorption Partitioning of Munitions Constituents to Soil** A. Miglino, R. Gonzalez, H.E. Allen, D.M. Di Toro, University of Delaware / Dept. of Civil & Environmental Engineering. It is a common observation that adsorption and desorption isotherms are not equivalent. A reversible – resistant component model has been proposed that separates the adsorption and desorption data into a reversibly sorbed component and a resistant component that does not desorb during the time span of the experiment. Adsorption and desorption of munitions constituents (MCs) on several soils with different properties exhibit reversible-resistant partitioning. The reversible/resistant model applied to our partitioning data indicates that a large fraction of many compounds are not recovered in typical desorption tests. A model, based on physicochemical processes will be presented that successfully reproduces the experimental results. The model assumes a rapid equilibrium between soil solution and a surficial soil layer and diffusive transport into the interior of the particle. Data for differing adsorption and desorption times and cycles of desorption are all successfully reproduced for RDX and nitroglycerin. The resistant component is due to the MC in the core of a soil particle that is trapped by partitioning and the slow mass transport due to the small aqueous concentration. Although the model assumes complete reversibility for the sorption process, the mass transfer-limited rate of release limits concentrations in soil solution, thus limiting the potential for risk.

**93 Energetic Contamination at Canadian Military Training Facilities: Updated Soil Concentrations for Military Training Sustainability (SCMTS)** P. Robidoux, National Research Council Canada / Aquatic and Crop Resource Development Portfolio, National Research Council Canada / Project Leader; Y. Berthelot, J. Dumas, S. Rocheleau, N. Besnier, V. Berube, National Research Council Canada; G.I. Sunahara, National Research Council Canada / NRC-Biotechnology Research Institute; J. Hawari, National Research Council Canada. Some operational activities of the Canadian Forces such as firing practice and munitions testing have led to the dispersion of energetic materials and metals in the Range and Training Areas (RTA). Sites contaminated by trinitrotoluene (TNT), hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX), 2,4-dinitrotoluene (2,4-DNT), 2,6-dinitrotoluene (2,6-DNT), and nitroglycerine (NG) may present a risk for the ecological receptors as well as to humans. The management of soils contaminated by these compounds on Canadian RTA is made on a case-by-case basis, using risk assessment methods, in the absence of generic soil quality guidelines. In order to simplify the environmental management of RTA, preliminary environmental and human health based Soil Concentrations for Military Training Sustainability (SCMTS) were developed in 2006 to support environmentally sustainable military training practices in Canada. Over the past years, ecotoxicity and bioaccumulation data have been generated. The SCMTS developed

for TNT, RDX, HMX, 2,4-DNT, 2,6-DNT, and NG have been recently updated. These new values and the results of ecotoxicological assessments using field collected soil and mesocosm studies used to validate the soil concentrations values will be discussed. Ecotoxicological assessment using field collected soils evaluated the effect of contaminated soils on direct contact organisms (plants, invertebrates) using standard parameters (lethality growth, reproduction) and selected sublethal parameters (biomarkers). Ecotoxicological assessment using mesocosms studies (field bioassays) evaluated the effect of contaminated soils on earthworms under field conditions using the lethality endpoint and validated sublethal parameters including immunotoxicity, oxidative and cellular stress responses.

**94 A case study of inter- and intra-species sensitivity differences to traditional and insensitive munitions** A.J. Kennedy, US Army Engineer Research and Development Center, CEERD-EPR; P. Gong, Specpro; S. Brasifield, J. Laird, ERDC; C. Lounds, Badger; M. Johnson, US Army Public Health Command; N. Barker, Badger; D. Gent, ERDC. The characterization for adverse environmental outcomes from discharging effluent is critical for understanding life cycle consequences for manufacturing. Aquatic toxicity is relevant to munitions production effluents discharged to surface water or waste water treatment facilities. However, the selection of test species or even populations within the same species may result in different toxicological thresholds. We employed laboratory populations of fish (*Pimephales promelas*) and cladocerans (*Ceriodaphnia dubia*, *Daphnia magna*) as well as both field and laboratory populations of the cladoceran *Daphnia pulex* to characterize the toxicity of a traditional munition (lead) and an insensitive munition (dinitroanisole, or DNAN). Insensitive munitions containing DNAN are proposed to replace trinitrotoluene (TNT) due to greater stability, safety and fewer restrictions on transport. While *P. promelas* is often more tolerant than cladocerans to many contaminants including lead, its sensitivity to DNAN was similar. Within one species (*D. pulex*), the expression of phenotypes such as fecundity and chemical sensitivity varied between laboratory and field genotypes. Further, variability within and between populations of *D. pulex* and associated selection of statistical (e.g., lowest and maximum allowable concentrations) versus point estimate (median lethal and inhibition concentrations) based toxicological endpoints resulted in a nearly 5-fold different threshold values. This reinforces the need for the multiple species approach and prior discussion and consensus on methods used for generating toxicity reference values for munitions. Despite this variability, the acute and chronic toxicity of DNAN was 14 and 9 fold less toxic than TNT, respectively, suggesting it may also be a more environmentally benign alternative. Further work involves confirming trends for additional *D. pulex* genotypes and determining a mechanistic explanation for differences in chemical sensitivity. Toxicological testing relying on a single species should be avoided to reduce the risk of misrepresentative information leading to incorrect or less economically feasible management alternatives when considering acquisitions or future clean up costs.

**95 Nano Particle Dispersion, Aggregation, and Bio-concentration within Whole Body and Target Organs of Aquatic Organisms** J.G. Coleman, US Army Engineer Research and Development Center, US Research and Development Center / Environmental Laboratory, US Army Engineer Research and Development Center / Research Biologist, US Research and Development Center / Research Biologist; A.J. Kennedy, US Army Engineer Research and Development Center, CEERD-EPR; J.D. Laird, US Army Engineer Research & Development Center / SpecPro Incorporated, U.S. Army Engineer Research & Development Center / Environmental Laboratory; A.J. Bednar, US Army Engineer Research and Development Center / Environmental Laboratory; J.F. Ranville, Colorado School of Mines / Chemistry and Geochemistry, Colorado School of Mines; A. Harmon, US Army Engineer Research and Development Center / Environmental Laboratory. As the production and applications of nanomaterials increase, it is essential to characterize the fate, transport, and effects in environmental systems and ecological receptor organisms. Our objective was to investigate the impact and target location of military-relevant nanoparticles within fish (*Pimephales promelas*, *Danio rerio*) and worms (*Lumbriculus variegatus*) following a direct, aquatic exposure. The present study utilized multiple detection instruments including single particle detection and field flow fractionation (FFF) coupled with inductively coupled plasma mass spectroscopy (ICP-MS), Dynamic Light Scattering (DLS), and CytoViva® hyperspectral microscopy to determine particle location, size, and concentration in treatments and target organs such as the gills, gut, and tissue. The extensive characterization

techniques were combined to assess potential for aggregation/dispersion within *in vivo* systems and detect target areas of bio-concentration. Treatment suspensions with various nanoparticles were created in serial dilutions of synthetic freshwater media with bath sonication (1 hour), *P. promelas* and *D. rerio* were exposed to 23 nm nano titanium dioxide (TiO<sub>2</sub>), 20-30 nm polyvinylpyrrolidone (PVP) coated nano silver, and 41 nm bovine serum albumin (BSA) nano gold for 96 hours at 25 ppm utilizing a continuous delivery system. *Lumbriculus variegatus* (n=3) were exposed to 30 nm and 70 nm PVP coated silver for 24 hours at a 5 ppm concentration. Post exposure, *L. variegatus* were depurated for 0, 8, 24, and 48 hours to assess relative gut residency time and potential to cross the digestive track into body tissue. In *D. rerio* and *P. promelas*, presence of nanoparticle aggregates was confirmed in the gut and gills of exposed fish. Particle retention in *L. variegatus* up to 48 hours was confirmed with the single particle detection-FFF and ICP-MS; hyperspectral imaging detected PVP particles within the gut compartment and potential translocation across the gut lining into tissue. Additionally, FFF of the *L. variegatus* suggested degradation of 70 nm PVP silver, resulting in a 50-60 nm particle, potential degradation mechanisms are being investigated. The proposed research will aid in determining bioaccumulation factors for nanomaterials within aquatic organisms and provide information about how these materials may enter the food chain through trophic transfer.

#### 96 Fate of Pb/Cu and 2,4-dinitrotoluene in Soil Slurries During Fenton Degradation

E. Jho, Seoul National University / Department of Civil and Environmental Engineering, Seoul National University; J. Jung, Seoul National University / Department of Civil and Environmental Engineering; K. Nam, Seoul National University / Department of Civil and Environmental Engineering, Seoul National University / Dept. of Civil and Environmental Engineering. Many contaminated sites such as firing ranges contain multiple contaminants such as heavy metals and organic contaminants. This study investigates the fate of heavy metals such as lead (Pb) and copper (Cu) and 2,4-dinitrotoluene (2,4-DNT) in soil slurries during modified Fenton reactions with lab-scale experiments. In the soil slurries (1:4 (w/v)) containing 2,4-DNT-only, 2,4-DNT-Pb, and 2,4-DNT-Cu, Fe(II)-citrate and a range of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) were added to initiate Fenton reactions. The addition of Fe(II)-citrate in the soil slurries, even in the presence of a large amount of Fe (37 g Fe kg<sup>-1</sup>) in the soil, increased the life-time of H<sub>2</sub>O<sub>2</sub> in the system. During 24 h Fenton reactions, the aqueous Pb mass decreased, while the aqueous Cu mass increased significantly resulting in almost complete mobilization of Cu. The residual 2,4-DNT in the aqueous and the soil phases decreased with increasing H<sub>2</sub>O<sub>2</sub> concentrations. The greater amount of reactive moieties produced and the production of reductive moieties for desorption at higher H<sub>2</sub>O<sub>2</sub> concentrations are likely the cause of such observation. Complete 2,4-DNT degradation in both aqueous and soil phases were achieved at 1 M H<sub>2</sub>O<sub>2</sub> in the 2,4-DNT-only and the 2,4-DNT-Cu systems; however, only ~80% of 2,4-DNT in both systems were degraded in the 2,4-DNT-Pb system. The lower 2,4-DNT degradation in the 2,4-DNT-Pb system could be attributed to the stronger binding of Pb on Fe oxide in soil than Cu, which leads to the reduced amount of available Fe for H<sub>2</sub>O<sub>2</sub> consumption and hence 2,4-DNT degradation. Interestingly, the 2,4-DNT degradation at lower H<sub>2</sub>O<sub>2</sub> concentrations was lower in the 2,4-DNT-Cu system, but higher at higher H<sub>2</sub>O<sub>2</sub> concentrations. This could be because Fenton-like reactions by Cu, which dominates the system over Fe at lower H<sub>2</sub>O<sub>2</sub> concentrations, degrade 2,4-DNT. With further increase in H<sub>2</sub>O<sub>2</sub> to 2 M in the 2,4-DNT-Pb system, further degradation of 2,4-DNT in soil phase was observed, while no enhancement was observed in the aqueous phase. The findings from this study suggest the prior removal of Pb and Cu before 2,4-DNT degradation by Fenton reactions and the use of higher H<sub>2</sub>O<sub>2</sub> concentrations (>1 M) for complete removal of 2,4-DNT from contaminated soils.

#### 97 Examination of Transport Mechanisms and Challenges of Chemical Fingerprinting of MC252 and Other Petroleum Sources in the Deep Sea Sediments

P.D Boehm, Exponent, Inc. / Environmental & EcoScience Group, Exponent / Environmental Group, Exponent, Inc. / Environmental & EcoScience Group; J.S. Brown, Exponent Inc.; L. Cook, Exponent. Extensive sampling and detailed chemical analyses (>1000 samples) of sediments and oil seeps within 20 miles of the Deepwater Horizon (Block MC252) wellhead and elsewhere have indicated that MC252 oil can be detected in sediments near the wellhead and that seep influences are pervasive in sediment in the area to the southwest of the wellhead over which the

physically and chemically dispersed oil in deepwater transited after release. The available data indicate that some naturally-occurring seeps oils in this study area, within 20 miles of the wellhead (especially around the Biloxi and Dauphin Domes) and fresh oil from MC252 are very similar in chemical composition – alkanes, polycyclic aromatics, saturated biomarkers (hopanes and steranes). Furthermore both seep oils and MC252 oil in the sediments appear to biodegrade similarly. As part of this biodegradation, the C27 steranes, previously thought to be highly resistant to biodegradation, are themselves biodegrading, thus altering sterane patterns and confounding the ability to distinguish sources (seeps and MC252) from one another. Thus the possibility of false positive identifications of sedimented MC252 oil is significant. Approaches to using the biodegradation patterns of steranes and other hydrocarbon compounds, degradation resistant biomarker ratios, and other chemical indicators to distinguish sources and to map the oil footprint will be discussed. The fingerprinting results along with the observed levels of hydrocarbons and PAHs in samples from the deep se area around the well are combined to discuss the conceptual model for transport of oil to the sediments. The vast majority of quantifiable MC-252 oil that reached the sediments in the offshore Gulf of Mexico did so through a single primary mechanism of deposition: adsorption of oil to heavy drilling muds and subsequent sedimentation as a consequence of the unsuccessful top kill effort. The area impacted by the top kill drilling mud deposition was limited and in close proximity to the Deepwater Horizon wellhead. To varying degrees, limited empirical evidence exists to support the plausibility of two secondary or isolated transport pathways for deposition of small amounts of MC-252 oil to the sediment – sorption onto particles/detritus; very minor and isolated sinking of *in situ* burn residues.

#### 98 MC252 oil spill post-incident finfish and shellfish PAH and dispersant (DoSS) tissue concentrations

G.R Mancini, E.R. Mancini & Associates; J. McCoy, Cardno Entrix; R. Barrick, Infinity Solutions Group; L. Elmore, Cardno Entrix. Total body burden tissue concentrations for total PAH (tPAH) and the dispersant compound dioctyl sodium sulfosuccinate (DoSS) were analyzed for six finfish and shellfish species (295 total specimens) collected from Louisiana shallow water coastal habitats in late 2010 (Oct. – Dec.) and mid-2011 (May – Aug.). Concentration data were developed for each species within each of seven state-established *Coastal Study Areas* (CSA 1 through 7) between the borders of TX and MS. Arithmetic mean tPAH concentrations for finfish and crustacean species ranged from 9.7–26.4 µg/Kg wwt while oyster concentrations ranged from 44.3–63.7 µg/Kg wwt across all CSAs. Mean concentrations were not significantly different between the reference CSA (CSA 7) and any other study area for four of the six species (anchovy, brown and white shrimp, and blue crab). For oyster and Atlantic croaker, statistical differences in mean tPAH concentrations were noted in only one and two CSAs, respectively. Mean tPAH tissue concentration data were also characterized according to “oiling categories” including reference condition [no known MC252 slick exposure], light exposure condition [1-20 days of documented slick exposure] and undetermined exposure condition, based on observed slick proximity to specimen capture locations. For two of the six species (Atlantic croaker and anchovy), the mean tissue concentrations from the reference area were slightly greater than the light exposure and undetermined exposure category tissue concentrations. For blue crab, brown shrimp, white shrimp, and oyster, tissue concentrations were within -1, -3, -2.5, and -4.5 µg/Kg wwt among the oiling categories, respectively. DoSS was detected in only 4 of 280 tissue samples (~1.4% of total; 79 µg/Kg dwt [blue crab]; 440 µg/Kg dwt [anchovy]; and 310 and 32 µg/Kg dwt [brown shrimp]) and these detected DoSS concentrations were less than established LOCs. In fact, for all tested species, documented tissue concentrations for tPAH and DoSS were orders of magnitude lower than *Levels of Concern* established by the Fish Consumption Advisory Group. The mean oyster tissue tPAH concentration documented in this study (430 µg/Kg dwt [Louisiana]) was also less than the mean Mussel Watch Program concentrations for oyster reported in either 1988 or 2008 (536 µg/Kg dwt [Gulf of Mexico] and 463 µg/Kg dwt [Louisiana], respectively).

#### 99 Photoenhanced Toxicity of WAF Oil to Early Lifestages of Gulf of Mexico Aquatic Species

A. Roberts, University of North Texas; J.T. Oris, Miami University / Department of Zoology, Miami University / Office for the Advancement of Research & Scholarship; M. Alloy, University of North Texas / Department of Biological Sciences; A. Tucker, Miami University; T. Curran, University of North Texas; D. Baxter, University of North Texas / Department of Biology, Institute of Applied Sciences; M. Grosell, RSMAS,



University of Miami; A. Volety, Florida Gulf Coast University; R. Griffin, University of Southern Mississippi; J.M. Morris, J. Lipton, D. Cacula, Stratus Consulting. The 2010 Deepwater Horizon Oil Spill resulted in a large release of polycyclic aromatic hydrocarbons (PAH) into the Gulf of Mexico. PAH exert a variety of effects on aquatic organisms including photoenhanced toxicity. Photoenhanced toxicity is a well described phenomenon in which molecules of certain PAH interact synergistically with ultraviolet radiation (UV). This interaction generates reactive oxygen species resulting in oxidative stress and acute toxicity. Early life stages of aquatic organisms are generally more susceptible to photoenhanced PAH toxicity than juvenile and adult stages. The purpose of this study was to assess the sensitivity of early life stages of Gulf of Mexico aquatic species to PAH photoenhanced toxicity. Test organisms included early life stages of blue crab (*Callinectes sapidus*), sheepshead minnow (*Cyprinodon variegatus*), and mahi-mahi (*Coryphaena hippurus*). Exposure media consisted of PAHs in water accommodated fractions prepared using slick or weathered source oil dispersed using a high energy method (HEWAF) or chemical dispersants (CEWAF). Four UV conditions were achieved using an outdoor microcosm exposure system to simulate high intensity light conditions and an indoor simulator to achieve low intensity light conditions. Survival was assessed as the endpoint for most species but hatching success was included for some life stages. Phototoxic effects on survival and hatching were observed in both a UV- and PAH concentration-dependent manner. These data may be used in models estimating site-specific risk for photoenhanced toxicity in the waters of the Gulf of Mexico.

**100 Nearshore Sediment Exposure to Louisiana Crude Oil: Spatial and Temporal Patterns in the Northern Gulf of Mexico** L. Riege, Cardno Entrix / Natural Resources; R. Dickey, C. Thomas, J. Brewer, S. Villanueva, Cardno Entrix. Sediments of the northern Gulf of Mexico are exposed to hydrocarbons from a variety of sources. In response to the Deepwater Horizon Oil Spill, BP and state and federal natural resource trustees are working cooperatively to gather data on potential injury from the spill to the nearshore resources during the Natural Resource Damage Assessment (NRDA). This talk summarizes the analysis of over 1,100 sediment samples collected as part of Response, NRDA cooperative and NRDA independent studies in 2010 and 2011 to provide the most complete picture possible of exposure to MC252 oil in the nearshore subtidal zone from the Texas/Louisiana boarder to Apalachee Bay, Florida. We report on spatial and temporal patterns of total PAH concentrations and petrogenic PAHs for the near-surface sediments and compare these results to the December 2010 Deepwater Horizon MC252 Operational Scientific Advisory Team (OSAT) report, providing updated information on the nature and distribution of spill-related hydrocarbons in the nearshore environment.

**101 Effects of exposure to oiled sediment from Barataria Bay, LA on the development of Gulf killifish (*Fundulus grandis*) embryos** F. Galvez, Louisiana State University / Dept. of Biological Sciences; C. Brown, Louisiana State University; A. Kuhl, LSU Aquaculture Research Station; C.C. Green, Louisiana State University / Renewable Natural Resources; J.A. Nyman, Louisiana State University / School of Renewable Natural Resources; A. Whitehead, University of California Davis; B.D. Dubansky, Louisiana State University / Department of Biological Sciences, Louisiana State University / graduate student. Preliminary data show that Gulf killifish (*Fundulus grandis*) sampled along Louisiana coastal marsh were impacted by the Deepwater Horizon (DWH) oil spill and displayed genomic responses that were predictive of toxicity and reproductive impairment, the latter of which is of particular concern. Marine fish embryos are highly sensitive to chemical components of weathered crude oil. Exposure can cause significant developmental defects, including cardiac dysmorphogenesis, which results in a cascade of pathologies including edema and hemorrhage. In the current study, developing killifish embryos were exposed to oiled water and sediments derived either from laboratory mesocosms, or collected from Louisiana marsh oiled by the DWH oil spill. Embryos exposed to sediments collected from oiled sites demonstrated delayed hatch rates, were smaller at hatch, and exhibited developmental abnormalities characteristic of exposure to the chemicals found in crude oil including cardiac dysmorphogenesis and associated pathologies. We are currently assessing the early-life stage effects on embryos exposed to sediments and water containing mixtures of crude oil and COREXIT dispersant at varying stages of weathering, and at varying salinities. Our overarching goal is to determine whether exposure to oil contaminated sediments will cause biological effects that emerge at the

population-level, and to characterize the spatial extent of such effects across the Louisiana coast of the northern Gulf of Mexico (nGOM).

**102 Post-Deepwater Horizon Toxicity Study of Gulf of Mexico Sediments using the Amphipod *Leptocheirus Plumulosus*** G.M. Huddleston III, ENTRIX, Cardno Entrix; A.D. McQueen, J. Kubitz, M. Cejas, Cardno Entrix; R. Barrick, Infinity Solutions; O.X. Pelz, BP Gulf Coast Restoration Organization. Toxicity tests were conducted on offshore sediment samples collected approximately one year after the Deepwater Horizon accident occurred. Samples were collected from May 25, 2011 to June 11, 2011 as part of a follow-up study of offshore and deepwater sediments (>200 m depth) to identify potential oil-related impacts on sediments and resident benthic fauna. A total of 38 sites were sampled, including: 17 sites within approximately 3 km of the MC 252 wellhead (1439-1607 m depth); 10 sites between 3 to 25 km of the wellhead (1025-1607 m depth); four sites beyond 25 km of the wellhead (1303-1516 m depth); and seven historic/pre-spill reference sites. Toxicity tests supplemented biological, chemical, and physical analyses of these sediments, which were collected using a multicorer (12 core system) device. Samples used in toxicity tests were composites of the top 3 cm of sediment cores from three replicate samples from 37 of the 38 stations. Static, 10-day bioassays using the amphipod *Leptocheirus plumulosus* (mortality and growth) were conducted to provide standard, naïve (non-tolerant) laboratory organism response to sediment exposures. Test organism mortality was statistically significant relative to laboratory controls (control sediment source: Lake Pontchartrain, Louisiana) in 12 of the 37 samples (ToxCalc 5.0.32  $p=0.05$ ). Within a 3-km radius of the wellhead, 10 of 17 samples exhibited significant mortality relative to laboratory controls. Between 3 and 25 km of the wellhead, only 1 of 10 samples exhibited significant mortality compared to laboratory controls. One sample beyond 25 km (approximately 62 km from wellhead) resulted in significant mortality relative to laboratory controls; however, evidence of drilling mud contamination unrelated to MC-252 was present. These results, along with other sediment information associated with this plan (e.g., grain size, chemical fingerprinting, and macrofauna and meiofauna data) help identify the spatial extent of MC-252 oil in sediments, and potential for associated injury to benthic biota.

**103 Development of oil product toxicity benchmarks using SSDs** M.G. Barron, US EPA / Gulf Ecology Division; C.R. Jackson, H.J. Michael, US EPA. Determining the sensitivity of a diversity of species to spilled oil and chemically dispersed oil continues to be a significant challenge in spill response and impact assessment. We developed species sensitivity distributions (SSDs) of acute toxicity values using standardized tests from the literature for several petroleum products and two Corexit oil dispersants that had data for ten or more aquatic species. Fifth percentile hazard concentrations (HC5s) were computed from the SSDs and used to assess relative oil product toxicity and to determine the feasibility of establishing toxicity benchmarks for a community of species. The sensitivity of mysids (*Americamysis bahia*) and silversides (*Menidia beryllina*) were evaluated within the SSDs to determine if these common test species were good surrogates for a broader range of species. In general, SSD development was limited by the availability of acute toxicity values that met standardization criteria for a diversity of species. Because there was only small variability in the HC5s for the compiled oils, pooled SSDs were also developed by oil type (e.g., crude, middle distillate). There was only limited variability in HC5 values determined from dispersant-only testing for the two Corexit products. The relative sensitivity of mysids and silversides varied across the oil products, with the toxicity values always greater than the HC5. Application of SSDs appears to be a reasonable approach to developing oil product toxicity benchmarks, but additional toxicity data are needed for a larger range of species conducted under standardized test conditions.

**104 Impacts of Deepwater Horizon Sub-Lethal PAH Exposure On Late Life Stage Pelagic Gamefish: Utilization Of Marine Finfish Aquaculture In Toxicology** J. Stieglitz, University of Miami, RSMAS / Department of Marine Biology and Fisheries, University of Miami – RSMAS; R. Hoenig, University of Miami, RSMAS; E. Mager, University of Miami / Rosenstiel School of Marine and Atmospheric Science, University of Miami, RSMAS / Division of Marine Biology & Fisheries; A. Esbaugh, University of Miami, RSMAS / Rosenstiel School of Marine and Atmospheric Science, University of Texas – Austin, Marine Science Institute / Post-Doctoral Fellow; D. Benetti, University of Miami, RSMAS; M. Grosell, RSMAS,

University of Miami. The Deepwater Horizon (DWH) oil spill in 2010 resulted in the release of approximately 200 million gallons of crude oil into the Gulf of Mexico (GOM). In addition, over 1.8 million gallons of dispersants (primarily Corexit) were used as part of the cleanup effort, with roughly 40% of this total applied at depth near the wellhead. The aim of the described research is to determine sub-lethal physiological impacts of polycyclic aromatic hydrocarbon (PAH) exposure on sub-adult and adult life stages of species representative of GOM finfish. Incorporation of advanced marine aquaculture technology to develop and maintain captive spawning broodstock populations of mahi-mahi (*Coryphaena hippurus*) and cobia (*Rachycentron canadum*) will be discussed, as well as techniques used to obtain blackfin tuna (*Thunnus atlanticus*) specimens for use in the toxicological bioassays. Preliminary tests have focused on establishment of baseline physiological data for the aforementioned species, including aerobic scope, maximum swim speed, and critical swim speeds ( $U_{crit}$ ). Sub-lethal acute PAH exposure tests are being implemented at the University of Miami to assess the aerobic scope and swim performance of the aforementioned pelagic gamefish utilizing swim chamber respirometry. Preliminary results indicate mahi-mahi possess superior maximum swim speeds as compared to blackfin tuna, as well as a greater aerobic scope. PAH-induced aerobic scope reduction is of particular concern for such high-performance species, due to potential subsequent reductions in migratory, feeding, fleeing abilities and vital physiological functions. Testing sub-lethal effects of acute PAH exposure on later life-stage specimens allows for elucidation of the potential impact of the DWH oil spill on high-performance and economically valuable pelagic gamefish.

**105 Estimation of the inventory of PBDEs in consumer products in municipalities adjacent the Great Lakes** G. Abbasi, University of Toronto / Geography and urban planning; M.L. Diamond, University of Toronto / Department of Geography and program in planning, University of Toronto / Department of Geography. Poly brominated diphenyl ethers (PBDEs) have been used as flame retardants in consumer products during the past few decades. Recent studies have revealed the rapid accumulation of widely used flame retardant chemicals in humans and wildlife, and new findings linked the human exposure to this class of chemicals to adverse health effects ranging from cancer to learning disabilities. Further the higher levels of PBDEs in the breast milk of women in North America as compared to other European countries, has raised public concern about the usage of these toxic chemicals in consumer products. Although these chemicals have been regulated or phased out in Canada since 2009 and in the United States since 2011, they have continued to be used in recycled products imported from other countries. These problems demonstrate a failure in both consumer product design and strategic policies to regulate toxic chemicals to protect human health and the environment. We believe that these initiatives are a direct response to the lack of adequate knowledge about the level of these hazardous substances in consumer products. Therefore our main goal is to quantify the human exposure to PBDEs based on their levels in in-use consumer products. Accordingly, we will estimate the current regional PBDE inventory based on two components: the inventory of PBDE-containing products and quantification of PBDEs contained within the inventory of products using Toronto and Chicago as two case studies. The result of this study will provide valuable metrics to quantify the present inventory of PBDEs in in-use products and anticipate the magnitude of PBDE reductions as a result of new regulations.

**106 Dechlorane Plus, a Chlorinated Flame Retardant, in the Great Lakes' Atmosphere** A. Salamova, Indiana University / School of Public and Environmental Affairs, Indiana University / SPEA; R.A. Hites, Indiana University / School of Public & Environmental Affairs,. Dechlorane Plus (DP,  $C_{18}H_{12}Cl_{12}$ ) is a highly chlorinated flame retardant that was introduced by Hooker Chemical (now known as OxyChem) in the 1960s as a replacement for Dechlorane (also known as Mirex). The technical DP mixture consists of *syn*- and *anti*-DP conformers in a ratio of 1:3 (i.e., the fraction of the *anti* isomer is 75%). DP is used as an additive flame retardant in wire and cable coatings, in hard plastic connectors used in televisions and computers, and in furniture. Although DP is a high production volume chemical and has been used for decades, it was first detected in the atmosphere, sediments, and fish of the North America Great Lakes only in 2006. After this finding, DP has been detected in air, sediments, precipitation, biota, house dust, tree bark, and humans in different parts of the world, suggesting that DP is a worldwide contaminant. Here we present temporal and spatial trends

in both *syn*-, *anti*-, and total DP concentrations in air (vapor and particle phases) and in precipitation at the five United States Integrated Atmospheric Deposition Network sites located in the Great Lakes basin. These samples were collected from January 2005 to December 2009, inclusive. The highest concentrations of these compounds were generally observed at the rural site at Sturgeon Point, New York, which is located near DP's manufacturing facility in Niagara Falls, New York, and at the urban site at Cleveland, Ohio. A multiple linear regression model was applied to the concentrations of these compounds in the vapor phase, particle phase, precipitation, and for the three phases combined. This regression resulted in an overall (three phases combined) doubling time of  $9.5 \pm 3.6$  years for the atmospheric concentrations of *anti*-DP, a clear indication of the continued use of DP.

**107 Contribution of Wastewater Treatment Plants to Concentrations of PBDEs, PFCs, PCBs, DDT, and Synthetic Musks in Fish Tissue from U.S. Urban Waters** J.B. Wathen, U.S. EPA Office of Water, Office of Science and Technology / Office of Science and Technology, U.S. EPA, Office of Water / Office of Science and Technology; L.L. Stahl, US EPA, Office of Water / Office of Science and Technology; J.M. Lazorchak, U.S. EPA, US Environmental Protection Agency / Office of Research and Development, U.S. EPA Office of Research and Development / National Exposure Research Laboratory, U.S. EPA / Molecular Indicators Research Branch, U.S. EPA / Molecular Ecology Research Branch; A.L. Batt, U.S. EPA Office of Research and Development / National Exposure Research Laboratory, US EPA; L. Osemwengie, U.S. EPA; A.R. Olsen, U.S. EPA Office of Research and Development / Western Ecology Division, National Health and Environmental Effects Laboratory; B.D. Snyder, Tetra Tech, Inc. / Center for Ecological Sciences. Wastewater treatment plants (WWTPs) are tasked with removing a wide variety of contaminants from influents, including BOD and nutrients from human waste as well as any and all other compounds that emanate from homes and commercial facilities in the communities they serve. Traces of these compounds, including PBDEs, PFCs, PCBs, DDT, and personal care products, have been found in fish tissues from U.S. rivers that are receiving waters for WWTPs. The extent to which organic contaminant compounds found to occur in fish tissue in may be attributable to facility discharges can depend on sources served by the collection system for the WWTP, the treatment system employed at the facility, and the chemical nature of the specific compound. We compared the concentrations of organic compounds analyzed in fish tissue relative to the location of the sampling point either upstream or proximally downstream of WWTPs at 160 statistically-representative urban water sites in U.S. rivers collected as part of EPA's National Rivers and Streams Assessment. The results of analyses of 124 water-column samples for 48 active pharmaceutical ingredients and 6 selected metabolites are also compared on the basis of their sampling locations relative to WWTPs. Preliminary analyses of data for PFCs did not suggest contributions from WWTPs, but sources for PBDEs are not well understood, PCB discharges likely are continuing from many WWTPs, and WWTP discharges are the likely source for synthetic musks.

**108 Estimating PBDE and PCB Loadings to the Lake Washington Watershed from Non-point Sources** J.A. Colton, King County Department of Natural Resources / Water and Land Resources Division, King Cty. Dept. of Nat. Resources / Water and Land Resources Division; R. Jack, King County / Department of Natural Resources. Polychlorinated biphenyl (PCB) and polybrominated diphenyl ether (PBDE) concentrations in Puget Sound harbor seal and orca blubber samples are some of the highest ever measured in marine mammals worldwide. Manufacture, sale and distribution of penta-, octa-, and some deca-PBDEs were banned in Washington State in January 2008 with a complete ban in January 2011. Like PCBs, PBDE sources in urban areas tend to be diffuse and non-point. The Lake Washington watershed is the most populous watershed in the state and considered a substantial source of contaminants to Puget Sound. However, data on PBTs in lake water and fish tissue are sparse. A fish consumption advisory is in effect on Lake Washington due to high tissue concentrations of PCBs; PBDE concentrations in fish tissue have not been examined. The goals of the current King County project are to quantify PBDE and PCB loadings to L. Washington and Puget Sound via the Ship Canal, identify the most important transport pathways needing PBDE and PCB loading reduction to the Lake Washington watershed, and quantify PBDE's bioaccumulating in lake fishes. This presentation reveals the first measurements of PBDEs in Lake Washington water and fish tissue as well as the first estimates of annual PCB and PBDE loadings from five major transport pathways into the



Lake. The input pathways studied were bulk air deposition, highway runoff, general stormwater runoff, CSO discharges, and major incoming streams. The fish species evaluated include smallmouth bass, northern pikeminnow, yellow perch, sculpin spp., and peamouth. This presentation focuses on the pre-modeling phase of an EPA-funded, 3-year project awarded to King County. Future project work will include fate and transport model and bioaccumulation model development. These models will enable prediction of improvements gained in fish tissue concentrations resulting from different loadings reduction scenarios.

**109 Is There an Unknown Exposure Pathway for PBDEs? Evidence from North American Biomonitoring Data** F. Wong, University of Toronto / Chemistry, Stockholm University / Applied Environmental Science (ITM); I. Cousins, M. Macleod, Stockholm University / Applied Environmental Science (ITM). The body burden of persistent chemical pollutants in members of the general population is determined jointly by the rate of intake of the chemical and the rate of removal from the body. We have examined this balance between intake and intrinsic elimination half-lives for polybrominated diphenyl ethers (PBDEs) in the North American population using the population-level pharmacokinetic model developed by Ritter et al. (2011). The model calculates the burden of chemicals in the bodies of representative members of the population as a function of age and calendar time from intake from all exposure pathways and a chemical-specific intrinsic elimination rate constant. The model can be fitted to empirical information from exposure and biomonitoring studies using a least-square optimization method. In this study, we collected empirical data from two studies that made total exposure estimates for the North American population for the years 2004 and 2005, and eight biomonitoring studies for the years 1992 to 2009. We assume intake of PBDEs increased exponentially to a peak in 2004, and has since been declining exponentially. The model fitting parameters are the intake of PBDEs in 2004 and 2038, and the intrinsic elimination rate constants, which can be expressed as equivalent half-lives. Fitting the model simultaneously with the biomonitoring and exposure data results in infinite elimination half-lives for all PBDE congeners. This result is not plausible since physical removal processes should limit the half-life of PBDEs to at most 15 years. Thus the modeling suggests that there is an inconsistency between the exposure studies and the biomonitoring data. When we calculate intakes using the biomonitoring data and elimination half-lives extrapolated to humans from a rat study we obtain intake estimates that are a factor of 5 higher than the empirical estimates for BDE-47 and -153. Even when we assume the maximum plausible elimination half-life of 15 years, intakes are still 2 and 5 times greater than empirical estimates for BDE-47 and -153 respectively. These intakes represent the plausible minimum that is required to explain the biomonitoring data. Our results thus indicate that a discrepancy between empirical exposure and biomonitoring studies exists, and that exposure of the North American population to PBDEs may have been underestimated in previous studies.

**110 Anthropogenic and naturally occurring halogenated phenolic compounds in the blood of Japanese terrestrial mammals** H. Mizukawa, K. Nomiyama, Ehime University; S. Nakatsu, Nakatsu Veterinary Surgery; S. Yachimori, Shikoku Institute of Natural History; T. Hayashi, Tochigi Prefectural Museum; Y. Tashiro, Meio University; M. Yamamoto, Y. Nagano, S. Tanabe, Ehime University. The present study elucidates the accumulation features of PCBs, PBDEs, their hydroxylated metabolites, and naturally occurring brominated compounds (methoxy PBDEs and bromophenols) in the blood samples of various carnivores such as cats, dogs, raccoon dogs, masked palm civets (MPC), foxes, raccoons, mongooses, and badgers collected from Japan. Moreover, the specific differences in metabolic capacities of different species for these compounds by analyzing the blood samples were investigated. Metabolic capacity for PCBs in terrestrial mammals was found to vary widely among species. Lower chlorinated OH-PCB congeners (3-5 Cl) were predominant in cat blood, whereas higher chlorinated OH-PCBs (6-8 Cl) were found in other carnivores. In particular, it can be presumed that cats may preferentially metabolize lower chlorinated PCBs and retain hydroxylated metabolites in their blood. The PBDE congener pattern in terrestrial mammals indicated a high proportion of BDE209, suggesting exposure to garbage and soil containing higher levels of deca-BDE products. The dominant congeners of OH-/MeO-PBDEs found in our study were 6OH-/MeO-BDE47 and 2'OH-/MeO-BDE68, which were already reported as natural products in the marine environment, accounted for up to 80% of total OH-/MeO-PBDEs in all the terrestrial mammals

investigated in this study. Moreover, higher accumulation of OH-PBDEs and MeO-PBDEs were found in cats than other carnivorous species, suggesting that cats may be intake of these compounds from food such as fish. Moreover, 2, 4, 6-tribromophenol, which was reported as both a natural compound and a man-made flame retardant, was dominant in all analyzed species except mongooses. Penta bromophenol and 2, 4, 5-tribromophenol were found in the blood of dogs, raccoons, foxes, MPCs, raccoon dogs, mongooses and cats, suggesting that these compounds could be the metabolites of PBDEs. As cats exhibit higher accumulation and a specific pattern of OH-PCBs, OH-PBDEs, and MeO-PBDEs, they may be at a high risk from these metabolites. To our knowledge, this study is the first report to comprehensively document the accumulation profiles of organohalogen metabolites and metabolic capacities in terrestrial mammals.

**111 Can hair be used to examine dose dependent pharmacokinetics of polybrominated diphenyl ethers (PBDE) in rat hair?** K. Aleksa, Hospital for Sick Children / Department of Clinical Pharmacology and Toxicology, University of Waterloo / School of Pharmacy, Hospital for Sick Children / Dept Clinical Pharmacology/Toxicology; A. Carnevale, University of Toronto; S. Ernest, McGill University; M. Wade, Health Canada; B. Hales, C. Goodyer, McGill University; G. Koren, Hospital for Sick Children. The ability to detect increasing levels of a xenobiotic in biological samples (e.g., blood or urine) has been long established thus enabling physicians to infer the amount of chemical present in the organism and the amount individuals was exposed to in their environment. However, there are times when obtaining these matrices can be difficult, such as in newborns, and there is a need to find an alternate matrix that can reflect long-term exposure and not just at the time of sampling. Hair offers a wider window for retrospective detection of chronic and past exposure/consumption of xenobiotics. Such information is commonly lacking in the case of environmental toxins. Polybrominated diphenyl ethers (PBDE) are implicated as endocrine-disrupters and are used worldwide in polymers incorporated into a variety of consumer products to prevent flammability. To determine if PBDEs are use full biomarkers of systemic exposure and whether a dose-response can be detected in hair, increasing amounts of BDEs were administered to rats and the hair was analyzed for the presence of BDEs. Methods: Adult Sprague Dawley rats were divided into 5 groups (n=10/group) and administered, by gavage, a BDE mixture (0, 0.02, 0.2, 2 or 20 mg/kg/day) mimicking house dust levels for 60 days. Thirty mg of hair was analyzed by GC/MS for BDE-28, 47, 99, 100, 153, 154, 183 and 209. Results: PBDEs were detected in all treatment groups (0.02 to 20 mg/kg/day). There was a clear dose response curve. The  $\Sigma$ PBDE was  $0.169 \pm 0.157$ ,  $0.265 \pm 0.089$ ,  $1.706 \pm 0.918$  and  $7.504 \pm 2.595$  ng/mg hair for 0.02, 0.2, 2 and 20 mg/kg/day treatments, respectively. The levels were statistically significant ( $p < 0.001$ ) from control for each group. BDE-28 was not detected in any treatment group; BDE-183 was present in only the 20mg/kg/day group and; BDE-209 was present in all groups including control ( $0.041 \pm 0.011$  ng/mg). BDE-47, 99, 100, 153, 154, and 209) levels increased with increasing levels of administered BDEs, with the levels being statistically significant ( $p < 0.001$ ) for the 2 and 20 mg/kg/day. Conclusion: BDE accumulates in a dose response manner. Variations may be due to differences in individual congener metabolism prior to incorporation. Although not all congeners showed a dose response in the hair, further work to determine the *in vitro* metabolic pathway is needed to determine why these differences are present.

**112 How Toxic Are Halogen-Free Flame Retardants? Aquatic toxicity of alternative flame retardants to Daphnia magna** S.L. Waaijers, University of Amsterdam / Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam/IBED Institute; M. Soeter, J. Hartmann, University of Amsterdam; S. Kools, Grontmij (Aquasense); M.H. Kraak, J.R. Parsons, W. Admiraal, P. de Voogt, University of Amsterdam. Several halogenated flame retardants have been banned due to their persistence, bioaccumulation potential, toxicity (PBT) and probable environmental risk. Hence, halogen-free flame retardants (HFFR) are required and a range of new flame retardants (FR) have been proposed. However, reviewing the literature we demonstrated that the PBT properties of most alternative flame retardants are poorly characterized. Therefore, the aim of the present study was to generate toxicity data for HFFRs by performing aquatic toxicity experiments with *Daphnia magna*. To this purpose daphnids were subjected to the selected compounds in 48 h immobility tests, based on OECD guideline 202. For each compound, first the concentration that equals water saturation (water solubility, Sw) was tested. Second, if an effect was observed at Sw, a dilution

range was prepared (4 concentrations per compound) and tested to determine the EC<sub>50</sub>. In addition, chronic toxicity experiments were performed, based on OECD guideline 211. The alternative flame retardants studied included six organic phosphates (aluminium diethyl phosphinate (ALPI), bisphenol a bis(diphenyl phosphate) (BDP), dihydro oxa phosphaphenanthrene (DOPO), melamine polyphosphate (MPP), resorcinol bis(diphenyl phosphate) (RDP), triphenyl phosphate (TPP)) and six inorganic compounds (aluminium trihydroxide (ATH), ammonium polyphosphate (APP), antimony trioxide (ATO), magnesium hydroxide (Mg(OH)<sub>2</sub>), zinc hydroxy stannate (ZHS) and zinc stannate (ZS)). Tetrabromobisphenol a (TBBPA) was tested as well, as a reference compound for halogenated flame retardants. All exposure concentrations are measured using ICP-OES, except for BDP, RDP, TPP & TBBPA, for which analysis is in progress. The brominated flame retardant exerted the highest toxicity during this study (EC<sub>50</sub> 50 = 1-10 mg/L). All other tested compounds showed low acute toxicity to *Daphnia magna* (EC<sub>50</sub> >10 mg/L, or EC<sub>0</sub> >Sw). Therefore, ALPI, BDP, DOPO, MPP, RDP, ATH, APP, Mg(OH)<sub>2</sub>, ZHS and ZS are considered here to be the most promising HFFRs. Chronic toxicity experiments currently in progress will reveal whether their toxicity will increase upon prolonged exposure. Our results contribute to the evaluation of the environmental safety of these HFFRs, and hence to their potential as alternatives for the halogenated flame retardants.

**113 Safety/toxicity assessment of ceria (a model engineered nanomaterial)** R. Yokel, University of Kentucky / Pharmaceutical Sciences; S. Hardas, A. Butterfield, R. Sultana, University of Kentucky / Chemistry; M. Dan, University of Kentucky / Pharmaceutical Sciences; J. Unrine, University of Kentucky; U. Graham, University of Kentucky / Center for Applied Energy Research; R. MacPhail, US EPA / Neurotoxicology Division; M. Tseng, University of Louisville / Anatomical Sciences and Neurobiology; P. Wu, E. Grulke, University of Kentucky / Chemical and Materials Engineering. Ceria engineered nanomaterial (ENM) is a catalyst, used in diesel fuel with potential in energy storage, and is an abrasive used in chemical-mechanical polishing. The long-term objective of this project is to investigate nanoceria physical-chemical properties that influence its distribution, biopersistence, and effects after introduction into the vascular system of the rat. To enable maximal understanding of the physical-chemical properties of the studied ENM, in-house synthesized, extensively characterized, citrate-coated, 5, 15, 30, and 55 nm cubic/polyhedral ceria and a ceria nanorod were intravenously infused into rats terminated 1 h to 90 days later. Cerium was quantified by ICP-MS in blood, plasma and blood cells; brain; peripheral tissues; urine and feces. Multiple oxidative stress markers were assessed. Ceria ENM distribution and cellular accumulation and histological changes were studied using light and electron microscopy. Valence of ceria ENM as prepared and *in situ* were determined using electron energy loss spectroscopy. Less than 1% of a 30 nm ceria dose was eliminated in feces and urine within 14 days, of which 98% was in feces. Five nm ceria clearance from blood was slower than the 15, 30, or 55 nm ceria ENM. Nanoceria increased in blood within a few h after its administration and in the liver and spleen from 1 h to 30 days, suggesting re-distribution from (an)other site(s), which might have been association with the vascular luminal wall. Ceria ENM accumulated in the mononuclear phagocyte system from which it was not significantly cleared over 90 days. Ceria ENM distribution following 5 doses that were 20% of a larger dose paralleled the larger dose, suggesting a large dose predicts the bioaccumulation of repeated small doses. Electron microscopy revealed very little ceria ENM in the brain, consistent with ICP-MS results. Initial anti-oxidant effects were temporally followed by pro-oxidant effects. Liver and spleen histopathology, including granulomas, observed 30 days after ceria ENM administration, did not abate or progress over 90 days. The surface valence of the ceria ENM, rich in Ce (III), did not significantly change after 30 days *in situ*. Nanoceria biopersistence, associated with toxicity, is consistent with the concern that slow clearance of insoluble nanomaterials may be result in adverse responses. Supported by US EPA STAR Grant Number RD-833772.

**114 Speciation and transformations of platinum in environmental materials** M.M. Shafer, University of Wisconsin-Madison / Environmental Chemistry & Technology, University of Wisconsin-Madison / Environmental Chemistry & Technology Dept.; J.J. Schauer, University of Wisconsin / Environmental Chemistry & Technology. Our research addresses three major questions: (a) what are the primary sources and environmental receptors of platinum and nano-sized platinum? (b) what are the chemical forms of

platinum introduced into the environment from current and potential major sources? and (c) how does the speciation of platinum change within specific environmental reservoirs after release? Our focus is on aerosol-mediated emissions, transport, and exposure in non-occupational settings. Emissions from vehicles are being addressed using roadside aerosol sampling and a synoptic program of roadway dust sampling. Engine dyno experiments were conducted to evaluate platinum emissions from platinum-cerium based fuel-borne catalysts (FBC). Concentrations and chemical speciation of platinum in particulate and "soluble" phases of these samples was determined with a suite of analytical tools. sXAS was applied to solid phases. "Soluble" species, as defined with physiological relevant fluid extractions including Gamble's Saline and Alveolar Macrophage Vacuole Fluid, are characterized for particle size (ultrafiltration), and charge (IC). The presence of the particularly toxic chloroplatinate species is being probed using an HPLC-IC-ICPMS method. Platinum species transformation will be evaluated in controlled laboratory experiments with both environmental and model samples. Road dust and road-side aerosol samples from cities across the country (including Atlanta, Denver, Los Angeles, Milwaukee) exhibit elevated levels of total platinum (200–800 ng/g). Significant (8–23% of total) soluble pools of platinum, with measurable anionic character, were measured in these vehicle emission receptor samples. Our sXAS studies of aerosol emissions (PM) from diesel engines burning a Pt/Ce-based FBC reveal a large fraction of oxidized platinum. Spectral fitting suggests that a platinum(IV)oxide is the dominant oxidized platinum species in the engine PM. Similarly, a substantial component of the platinum pool in used TWCs was found to be oxidized. The majority of the primary emissions of platinum from diesel engines burning a Pt-FBC was present in fine and ultra-fine particle-size fractions. We have advanced the analytical methodology for separation and detection of hexa- and tetra-chloroplatinate to achieve quantification limits an order-of-magnitude better than reported in the literature.

**115 Environmental Behavior and Bioavailability of Ag and CeO<sub>2</sub> Nanoparticles: surface functionalization and interaction with natural organic substances** J.M. Unrine, University of Kentucky, University of Kentucky / Department of Plant and Soil Sciences, University of Kentucky / Department of Plant & Soil Sciences, University of Kentucky / Savannah River Ecology Laboratory, University of Kentucky / Department of Plant and Soil Sciences, University of Georgia / Savannah River Ecology Laboratory; E. Oostveen, B. Collin, University of Kentucky; O.V. Tsyusko, University of Kentucky / Department of Plant & Soil Sciences; U. Graham, University of Kentucky / Center for Applied Energy Research; D.A. Butterfield, University of Kentucky / Department of Chemistry; P.M. Bertsch, University of Kentucky / Department of Plant and Soil Sciences. The objective of this project is to investigate the role of nanoparticle surface chemistry and environmental modifications of nanoparticle surfaces in determining their aggregation, deposition and dissolution in environmental matrices, as well as bioavailability and toxicity to a model organism (*Caenorhabditis elegans*). We have synthesized CeO<sub>2</sub> nanoparticles (NPs) with a mean geometric diameter of 4 nm and a 10 kDa dextran coating (DEX). This coating was then functionalized to confer either a net positive charge (diethylaminoethyl dextran; DEAE), or a negative charge (carboxymethyl dextran; CM). The DEX-CeO<sub>2</sub> NPs are amphoteric and have a point of zero net charge (ZPC) of around 7, while the CM dextran coated particles display a net negative charge over a wide pH range and the DEAE particles have a net positive charge over a wide pH range. We are examining the environmental behavior of these particles, including interactions with natural organic matter (NOM), as well as their bioavailability and toxicity and how toxicity and bioavailability are modified by NOM. We observe clear changes in surface chemistry in exposure media such as moderately hard reconstituted water for the DEX CeO<sub>2</sub> NPs but not the DEAE CeO<sub>2</sub> NPs. Similarly, the ZPC of the DEX particles is shifted by up to 2 pH units upon interaction with NOM, but the ZPC of the DEAE particles is relatively unaffected. The positively charged DEAE CeO<sub>2</sub> NPs are far more toxic to *C. elegans* than the CM CeO<sub>2</sub> NPs which are more toxic than the neutral DEX CeO<sub>2</sub> NPs. These differences seem to be attributed to differences in bioavailability. Both the DEX and DEAE coated particles are mostly oxidized as prepared, with some Ce (III) on the surface of the particles. Upon interaction with *C. elegans*, the particles are reduced and contain up to 80% Ce (III). This suggests that reduction of Ce coupled with oxidation of biomolecules as a possible mechanism of toxicity. We intend to use redox proteomics and real-time PCR to help elucidate these mechanisms. In a separate study, we examined the effects of exudates released from aquatic plants (*Potamogeton*



*diversifolius* and *Egeria densa*) on the aggregation and dissolution of Ag NPs with either polyvinylpyrrolidone (PVP) or gum arabic (GA) coatings. The exudates oxidized and dissolved the GA Ag NPs, but stabilized and prevented aggregation of the PVP Ag NPs.

**116 Impact of Nanosilver and Silver Ions on Methanogenesis and Methane Production from Municipal Solid Waste** Y. Yang, S. Gajraj, University of Missouri / Civil and Environmental Engineering; J. Wall, University of Missouri / Biochemistry; Z. Hu, University of Missouri / Civil and Environmental Engineering. Silver nanoparticles (AgNPs, nanosilver) released from industrial activities and consumer products may be disposed directly or indirectly in sanitary landfills. To determine the impact of AgNPs on anaerobic digestion of landfill waste, municipal solid waste (MSW) was loaded in identical landfill bioreactors (9 L volume each) and exposed to AgNPs (average particle size = 21 nm) or silver ions. The landfill anaerobic digestion was carried out for more than 200 days, during which time the cumulative biogas production was recorded automatically and the chemical property changes of leachates were analyzed. There was no significant difference in the cumulative methane volume or methane production rate between the silver ions treated group and the control group. There was also no significant difference between the groups of control and 1 mg AgNPs/kg. However, landfill solids exposed to AgNPs at 10 mg/kg resulted in the reduced biogas production, the accumulation of volatile fatty acids (including acetic acid), and the prolonged period of low leachate pH (between 5 and 6). This was accompanied by higher average total Ag concentration of  $3.9 \pm 0.1$  mg/L in the leachates as compared to  $0.2 \pm 0.2$  and  $1.3 \pm 0.1$  mg/L for the groups of control and the bioreactor treated with 10 mg Ag<sup>+</sup>/kg, respectively. Quantitative PCR targeting 16S rRNA genes of methanogens indicated a slower methanogen growth rate in the bioreactor treated with 10 mg AgNPs/kg. The results suggest that at the concentration of 10 mg/kg, silver ions have minimal impact on landfill methane production. Nanosilver is more toxic than its counterpart resulting in reduced methane production and methanogenic assemblages, likely because of higher silver concentration available in the leachate due to the slow or minimal nanosilver dissolution under landfill operations.

**117 Photochemical and Fungal Transformations of Carbon Nanotubes in the Environment** C.T. Jafvert, S. BeigzadehMilani, Purdue University / School of Civil Engineering; D.H. Fairbrother, J.L. Bitter, Johns Hopkins University / Department of Chemistry; T.R. Filley, T. Berry, C. Gibson, Purdue University / Department of Earth and Atmospheric Sciences. Transformations of carbon nanotubes (CNTs) in the environment are likely to be dominated by abiotic oxidative and extracellular microbial processes. Consequently, we are investigating photochemical and fungal mediated transformations of CNTs, both individually and sequentially. Types of CNTs investigated include pristine (unfunctionalized) single-walled carbon nanotubes (SWCNT) from Carbon Solutions (AP and P2) and from SouthWest Nano Technologies (SG-65); and functionalized single-walled nanotubes from Carbon Solutions (P3 and P7). Also studied are SG-65 nanotubes and multi-walled nanotubes from NanoLab Inc that have been functionalized by acid treatment and further purified to remove metal catalysts (used in their preparation). Initial results indicate the recalcitrance of unfunctionalized SWCNTs to both solar light (after weeks) and fungal reactions. When suspended in water and exposed to solar spectrum light, both carboxylated and PEGylated SWCNTs produce reactive oxygen species (ROS). Singlet oxygen and superoxide anion have been detected from these two SWCNTs. Fungal species, *Trametes versicolor* and *Gloeophyllum trabeum* are currently being investigated for toxicity responses to the suite of CNTs in this study and show impeded growth in culture media when exposed to some unpurified CNTs. Because reaction may lead to further oxidation of carboxyl carbon, the rate of CO<sub>2</sub> evolution is being assessed in both sunlight irradiated samples and CNT preparations exposed to white and brown rot-type wood decay fungi. As a result of exposure to 254 nm light (a positive control for potential product identification), flocculation was observed for oxidized MWCNTs and SWNTs over a range of pH values (4-10), although their ability to resist aggregation was enhanced as the pH and the degree of CNT surface oxygen increased. The rate of photo-induced aggregation was found to depend linearly on the photon flux. Collectively, these observations are consistent with the idea that photo-decarboxylation of the oxidized CNTs is responsible for the effects of UV-irradiation. This hypothesis is currently being tested by using XPS in conjunction with

chemical derivatization to assay the change in oxygen concentration and functional group distributions induced by photolysis in both the UVC and solar regions.

**118 TiO<sub>2</sub> Nanoparticle Exposure and Illumination During Zebrafish Development: Mortality at Parts per Billion Concentrations** W. Heide-man, University of Wisconsin / School of Pharmacy; O. Bar-Ilan, University of Wisconsin / Division of Pharmaceutical Sciences; R. Peterson, J. Pedersen, H. Robert, University of Wisconsin. Titanium dioxide nanoparticles (TiO<sub>2</sub>NPs) can be activated by photons to produce reactive oxygen species (ROS). Exposure to TiO<sub>2</sub>NPs and illumination has the potential to produce cumulative cellular damage. To test this, we exposed zebrafish (*Danio rerio*) to two commercial TiO<sub>2</sub>NP preparations at concentrations ranging from 0.01 to 10,000 ng/ml over a 23-day period. This short period spans all of embryogenesis, larval development, and metamorphosis into the juvenile/adult body plan. During this exposure, some groups of exposed fish were also illuminated with a metal halide lamp that simulates solar irradiation below the surface of shallow water. TiO<sub>2</sub>NP exposure produced significant mortality at 1 ng/ml. As expected, this toxicity was photo-dependent. The LC<sub>50</sub> values for the two TiO<sub>2</sub>NP preparations were 14 ng/mL (95% CI: 0-400), and 268 ng/mL (95% CI: 50-2803). Toxicity included stunted growth, delayed metamorphosis, malformations, organ pathology, and DNA damage. TiO<sub>2</sub>NPs were found in the gills and gut, and elsewhere. Toxic responses were observed in gills, liver, and skin—where exposure to TiO<sub>2</sub>NPs is highest. The two preparations differed in particle diameter ( $12.1 \pm 3.7$  nm and  $23.3 \pm 9.8$  nm), but produced aggregates in the 1  $\mu$ m range, and both were taken up to approximately the same extent, in a dose-dependent manner. Illuminated particles produced a time- and dose-dependent increase in 8-hydroxy-2'-deoxyguanosine DNA adducts, consistent with cumulative ROS damage. Zebrafish take up TiO<sub>2</sub>NPs from the aqueous environment even at low ng/ml concentrations, and these particles, when illuminated in the violet-near UV range produce cumulative toxicity.

**119 Transformation and Fate of Nanomaterials During Wastewater Treatment and Incineration** L.C. Marr, Virginia Tech / Civil and Environmental Engineering; A. Pruden, Virginia Tech / Via Department of Civil and Environmental Engineering, Virginia Tech / Civil & Environmental Engineering; E. Vejerano, Y. Ma, A.L. Holder, Virginia Tech / Civil and Environmental Engineering. Disposal is the final step in the product life cycle, and understanding how waste treatment processes might modify nanomaterials is critical to predicting their fate in the environment. The overall objective of this research is to characterize the transformation and fate of engineered nanomaterials and byproducts during biological wastewater treatment and incineration. We have assessed the response of a nitrifying wastewater treatment system to nanosilver and titanium dioxide. The majority of the nanomaterials partitioned to the sludge in lab-scale sequencing batch reactors. Results of RT-qPCR indicated that nitrite-oxidizing bacteria were significantly reduced in reactors containing nanosilver compared those containing ionic silver and the control. The expression of specific tetracycline and sulfonamide antibiotic resistance genes did not change in response to the addition of nanosilver or ionic silver. We have characterized the microbial community through high-throughput pyrosequencing and used the Comet Assay to assess toxicity of the effluent. Incineration was simulated through combustion of waste samples spiked with 0.1%, 1%, and 10% by weight ceria, C<sub>60</sub>-fullerene, nickel oxide, silver, or titania in a muffle furnace. The presence of nanomaterials in the waste stream either enhanced or reduced emissions of polycyclic aromatic hydrocarbons (PAH), depending on the type of waste and the specific PAH. The most abundant PAH species found in the exhaust were those containing 2-3 rings, and their yields depended on the concentration of nanomaterials in the waste. Phenanthrene, fluorene, and anthracene were the dominant PAH species at nanomaterial concentrations of 0.1%, 1%, and 10%, respectively. Nanomaterials in the waste resulted in increased emissions of some of the 4-6 ring PAHs: benzo(k)fluoranthene, pyrene, and chrysene. The total PAH yields for the samples containing nanomaterials were ~6 times higher, on average, than for samples containing the nanomaterials' bulk counterparts, suggesting that the high specific surface area of the nanomaterials affects PAH formation. The shift in emissions occurred mainly in the 2-3 ring PAHs and not in the larger ones. Partitioning between the ash and airborne particulate matter was assessed for the metallic nanoparticles. At most, 3% of the nanomaterials were found in particulate matter.

**120 Fate and behaviour of silver nanoparticles in aquatic systems** J. Lead, University of Birmingham / School of Geography, Earth and Environmental Scien. Silver nanoparticles are one of the most used nanoparticles types due to anti-bacterial properties and such usage inevitably leads to environmental discharge. However, both the nature and extent of silver nanoparticle exposure and hazard are poorly understood. This talk will particularly focus on the potential environmental transformations of nanoparticles, related to aggregation, oxidation, dissolution and the formation of nanoscale coatings of natural organic macromolecules (NOM). These processes will be examined in relation to nanoparticle properties (size, capping agent) and environmental conditions (concentration and nature of NOM, pH, ionic strength, presence of specific ions). Speculations on potential timescales and ultimate fates of the nanoparticles will be given.

**121 Bioaccumulation of Emerging Contaminants in Tropical Mangrove Food Webs** B.C. Kelly, H. Zhang, F. Chen, National University of Singapore / Department of Civil and Environmental Engineering. Tropical mangroves are important ecosystems in Southeast Asia, as well as other tropical and subtropical regions of the world. These unique ecosystems provide essential habitat for numerous species of aquatic and terrestrial organisms. This paper reports results of a field study involving (i) stable nitrogen  $\delta^{15}\text{N}$  and carbon  $\delta^{13}\text{C}$  isotope analyses to assess nutrient dynamics and trophic position of mangrove organisms and (ii) trace chemical analyses to assess the bioaccumulation behavior of emerging contaminants of concern in mangrove ecosystems. Field sampling was conducted at two local mangroves in Singapore and included collection of water, sediments, suspended sediments, polychaetes, bivalves, crustaceans and fish. Stable isotopes were determined using isotope ratio mass spectrometry (IRMS). Target analytes included legacy POPs, as well as various contaminants of emerging concern including current-use pesticides (CUPs), brominated flame retardants (BFRs), synthetic musks and pharmaceuticals and personal care products (PPCPs). Following standard extraction and cleanup methods, identification and quantification of target compounds was performed using liquid chromatography-electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) or gas chromatography triple-quadrupole tandem mass spectrometry (GC-QQQ-MS/MS). Residues of PPCPs, Caffeine, Carbamazepine, Diclofenac, Diphenhydramine, Estrone (E1), Estradiol (E2), Gemfibrozil, Ibuprofen, Linuron, Naproxen, Simvastatin and Triclosan, were detected at levels of  $< 0.1$  to  $200 \text{ ng/g dry wt.}$  in sediments and  $< 0.1$  to  $20 \text{ ng/g wet wt.}$  in biota. Four synthetic musks (Celestolide, Galaxolide, Tonalide and Musk Ketone) were also commonly detected in mangrove samples. Galaxolide and Triclosan Metabolite (Methyl Triclosan) were detected at relatively high concentrations in biota ( $100$  to  $2500 \text{ ng/g lipid wt.}$ ). Observed species-specific bioaccumulation factors (BAFs), biota-sediment accumulation factors (BSAFs) and overall trophic magnification factors (TMFs) emerging contaminants were generally lower than those for legacy POPs. Bioaccumulation metrics of many emerging contaminants were lower than anticipated based on physical-chemical properties (e.g., chemical  $K_{OW}$  and  $K_{OA}$ ), indicating many of these compounds are likely metabolized in mangrove organisms.

**122 Derivation of critical body burdens to assess the potential risks of perfluorinated compounds to aquatic organisms** J. Newsted, Cardno Entrix, Cardno ENTRIX, Inc.. Perfluoroalkyl compounds (PFCs) are synthetic fluorinated organic compounds that can be released to the environment during manufacturing processes, from commercial products and applications, or indirectly via oxidation of precursor molecules containing perfluoroalkyl chains. While PFCs have received increased attention in monitoring programs, the assessment of the potential risks PFCs pose to aquatic organisms are still being investigated. The tissue residue approach (TRA) provides a framework for analyzing aquatic toxicity in terms of mode of action and tissue residue concentrations and was applied to perfluorooctane sulfonate (PFOS), PFC that has been extensively studied in laboratory and field studies. Using acute and chronic toxicity data from invertebrate and fish studies, critical body burdens were derived using several methods. In the first method, an assumption of steady state conditions was used along with bioaccumulation factors and whole body critical tissue levels were calculated using LC10 and LC50 values from freshwater organisms. Critical body burdens ranged from  $0.15$  to greater than  $60 \text{ mmol/kg ww}$  while tissue values based on MATC values ranged from  $0.03$  to  $13 \text{ mmol/kg ww}$ . Given the uncertainty related to the assumption of steady state conditions for acute and subacute exposures, a second analysis was conducted using

pharmacokinetic models in several aquatic organisms. In one example, a kinetically derived tissue residue value in bluegill was  $0.24 \text{ mmol/kg}$ . In a third approach, measured tissue values from toxicity study conducted with bluegill had a tissue value of  $0.47 \text{ mmol/kg}$ . While a critical body burdens for aquatic organisms can be derived for PFOS, the presence of significant data gaps introduce great amounts of uncertainty that at this time are difficult to address due to a clear understanding of the mechanisms of accumulation of PFOS into biota. However, given these constraints, this work presents an approach that can be used to further define critical body burdens for aquatic organisms. Finally, the results of a hazard assessment will be presented that compares the PFOS critical body burden values to PFOS concentrations in fish tissues collected from North America and other global locations.

**123 Dynamics of uptake and elimination of  $17\alpha$ -ethinylestradiol in male goldfish (*Carassius auratus*)** A. Alansari, University of Ottawa / Biology; J.M. Doyle, V.M. Trudeau, J.M. Blais, University of Ottawa. To determine the uptake and elimination kinetics of  $17\alpha$ -ethinylestradiol (EE2), goldfish (*Carassius auratus*) were exposed to EE2 via two different routes: water ( $150 \text{ ng/L}$ , nominal) for  $72 \text{ h}$  and food ( $250 \text{ ng/g}$ , nominal) for  $120 \text{ h}$ . Whole tissue EE2 levels revealed a rapid uptake of EE2 in goldfish, and reached  $8.43 \text{ ng/g}$  ( $\pm 1.22$ , Standard deviation, dry weight) after the first hour of exposure with an uptake rate constant ( $k_1$ ) of  $45 \text{ h}^{-1}$ . The elimination rate constant ( $k_2$ ) was  $0.0786 \text{ h}^{-1}$ . In the feeding experiment, EE2 concentrations increased rapidly, reaching an average of  $1.4 \text{ ng/g}$  after  $3 \text{ h}$  following the first dietary exposure with estimated assimilation efficiency ( $\alpha$ ) of  $0.106$ . An average of  $(2.66 \pm 0.87 \text{ ng/g, } n=8)$  was determined in the  $24$ – $72 \text{ h}$  samples, whereas EE2 concentrations in fish were below our method detection limits ( $0.67 \text{ ng/g}$ ) for all samples after  $72 \text{ h}$ . The calculated EE2 bioconcentration factor (BCF) for the water exposure was  $377$ . A good fit was observed between the experimental and modeled data. The modeling was critical to predict the extent by which EE2 bioaccumulation would occur at variable environmental concentrations. Our data suggested that EE2 can be rapidly taken up by fish via gill absorption and gastrointestinal absorption. To our knowledge, this was the first report to describe EE2 uptake and elimination dynamics, as well as providing important data to model EE2 kinetics following both water and dietary exposures in goldfish. Funded by NSERC-Canada and King Abdulaziz University, Saudi Arabia.

**124 Histopathological changes and bioaccumulation of platinum group metals by native Hudson River aquatic organism, *Orconectes virilis*** M. Wren, T. DesMarais, Marist College; Z. Gagnon, Marist College / School of Science. Platinum Group Metals (PGM), such as platinum (Pt), palladium (Pd), and rhodium (Rh), are of increasing concern due to rising anthropogenic input to aquatic systems. Most of United States' PGM consumption goes towards manufacturing catalytic converters. Current research demonstrates catalytic converter emissions' solubility in water and reactivity in exposed biota. Hudson River crayfish are at risk of PGM exposure as areas near the river become more densely populated and the number of automobiles increases. Crayfish have been recognized for their ability to absorb and deposit metals in their gills and exoskeleton, warranting them as an effective experimental model for metal accumulation. In this study, PGMs' effects on bioaccumulation and histopathological changes were studied using *Orconectes virilis*, a native species of the Hudson River, as a model. Raw Hudson River water was used to recreate the interactions seen between PGMs and existing chemicals polluting the water body. Organisms were exposed to varying experimental levels of water-soluble PGM salts for ten days. Experimental treatments were established:  $0.0$ ,  $1.0$ ,  $5.0$ ,  $10.0 \text{ ppm Pt}^{(IV)}$  and a PGM Mix ( $1.0 \text{ ppm Pt}^{(IV)}$ ,  $\text{Rh}^{(III)}$ ,  $\text{Pd}^{(II)}$  each). A Solar AA spectrometer in graphite furnace mode (GFAAS) was used to measure concentrations of metals in samples of exoskeleton, muscle, and liver. SPSS statistical package was used to determine differences in accumulation of Pt and Rh in crayfish tissue. Statistically significant bioaccumulation of Pt and Rh was observed in all organs, with highest concentrations found in exoskeletal tissue,  $1,856.6 \text{ } \mu\text{g}/0.1 \text{ g dw}$  in  $5 \text{ ppm}$  exposure and  $476.2 \text{ } \mu\text{g}/0.1 \text{ g dw}$  Rh in PGM mix exposure. Pt concentrations dropped significantly at  $10 \text{ ppm}$  Pt, suggesting that severe structural damage to the tissue disabled significant retention of the substrate. Paraffin sections,  $6 \text{ } \mu\text{m}$  thick, were prepared in eosin-hematoxylin stain and examined for histological abnormalities within liver and exoskeletal tissue. Inclusion bodies and hyalinization were observed in exposed liver tissue. Pathology in hepatocyte structure, such as hyper-segmentation of vacuoles and swelling of the vascular channels, were

also observed. Visible bands of the exocuticle and a lighter stain suggests demineralization in exposed exoskeletal tissue. Bioaccumulation and cellular abnormalities seen in exposed aquatic organisms raise concern of PGM biomagnification within the food chain.

**125 Internal concentrations of polychlorinated dibenzo-p-dioxins in the reporter gene assay CAFLUX for activation of the arylhydrocarbon receptor** B.I. Escher, Entox, The University of Queensland / National Research Centre for Environmental Toxicology; B. Mewburn, Entox, The University of Queensland; M.S. Denison, University of California, Davis / Department of Environmental Toxicology; J.L. Hermens, IRAS, Utrecht University. Persistent organic pollutants are ubiquitous environmental contaminants and pose health risks. Cell-based bioassays could be cost-efficient and ethical alternatives to animal testing in chemical risk assessment. However, hydrophobic organic chemicals may appear less potent than they really are because their bioavailability is reduced by sorption to growth medium and vials. In the present study we use the CAFLUX assay, CAFLUX assay uses a recombinant rat hepatoma (H4IIE) cell line containing a stably transfected AhR-inducible green fluorescent protein reporter plasmid. The CAFLUX is specific for chemicals binding to the aryl hydrocarbon receptor and detects chemicals with a dioxin-like activity. For a series of polychlorinated dibenzo-p-dioxins we have assessed bioavailability and partitioning between medium components and cells and demonstrate how the partitioning affects the cellular concentrations with 95% to more than 99% of the chemicals sorbed to medium components thus reducing the sensitivity of the assay. We also demonstrate how to overcome this limitation with a novel dosing technique where the polychlorinated dibenzo-p-dioxins are desorbed from a polymer. Depletion of free concentration by cells and medium components is replenished from polymer reservoir, thus keeping the aqueous and cellular concentrations constant over the duration of the experiment. While this approach is more precise than dosing by solvent spiking, it has the disadvantage that larger amounts of sample are needed due to the larger medium volume and the additional PDMS reservoir. Similar to PDMS, the proteins in the medium can keep the concentrations constant over the duration of the experiment and this might suffice for practical applications of environmental samples.

**126 Occurrence of Currently Used Pesticides in Tissues of Non-Target Organisms** K.L. Smalling, K.M. Kuivila, U.S. Geological Survey. A wide variety of pesticides are applied to agricultural and urban areas throughout the United States and are transported off-site either dissolved in water, bound to sediments, or by aerial drift during application. The effects of these current-use pesticides on aquatic ecosystem health are not well understood. Pesticide residues in tissues of non-target organisms demonstrate exposure and may serve as a metric that can be linked to biological endpoints. Studies of legacy pollutants have shown that tissue concentrations are potentially a better surrogate for characterizing toxicity to non-target organisms than traditional toxicity tests using external exposure concentrations (water, sediment, diet). Relatively little data are available on the occurrence of current-use pesticides in non-target organisms, and thus, linkages between tissue concentration and toxicological endpoints are rarely established for these compounds. To better assess potential ecosystem effects, the first step is to characterize pesticide exposure to aquatic organisms of concern by directly measuring concentrations in tissue. Because organisms are typically exposed to complex mixtures of pesticides, a relatively recent method was developed to measure approximately 100 pesticides and degradates in tissue (either whole body or individual organs). The most statistically significant approach to correlate pesticide exposure and effects is to measure both tissue concentration and biological endpoints (lethal and/or sublethal effects) in individual organisms (rather than composite samples). For this reason, the analytical method was optimized for maximum sensitivity to allow for the analysis of currently used pesticides in single organisms or organs. A variety of tissues, including fish, frogs, sand crabs, crab embryos, and insect larvae, from sites across the United States were analyzed for current-use pesticides. Results indicate that organophosphate and pyrethroid insecticides, conazole and strobilurin fungicides, and a variety of herbicides are present at measurable concentrations in tissues from a variety of organisms residing in substantially different habitats. Future studies will include collaborative efforts between chemists, toxicologists, and ecologists to link observed tissue pesticide concentrations to aquatic ecosystem health.

**127 Uptake and trophic transfer of perchlorate in a simulated northern pike and threespine stickleback food web** C. Furin, University of Alaska Anchorage / Dept. of Biological Sciences; F.A. von Hippel, University of Alaska Anchorage / Department of Biological Sciences; B. Hagedorn, University of Alaska, Anchorage / Environment and Natural Resources Institute; T. O'Hara, University of Alaska Fairbanks / Institute of Arctic Biology. The uptake and trophic transfer of contaminants in aquatic food webs is a major concern. This study addresses the ability of the environmental contaminant perchlorate, a highly water-soluble endocrine disruptor, to 1) biomagnify (food) and/or bioconcentrate (water), or not; and 2) assesses tissue concentrations for each route alone and both routes combined. We investigated how different routes of exposure (food and/or water) affect tissue concentrations by exposing threespine stickleback (*Gasterosteus aculeatus*) to perchlorate and then feeding them to northern pike (*Esox lucius*) housed in different water concentrations. Perchlorate exposure to the pike were through contaminated water (49 day exposure at either 10 ppm or 100 ppm) and/or food (14 day exposure to stickleback prey previously held in water at either 10 ppm or 100 ppm). We found that both water and food significantly contribute to tissue concentrations as compared to controls, but that, as expected, perchlorate does not biomagnify. Contaminated food contributed to pike tissue concentration in the control and 10 ppm water treatments but did not in the 100 ppm water treatment. These results demonstrate that assessing the combined route of exposures (food and water) is important to consider when evaluating uptake and tissue concentrations of perchlorate in fish.

**128 Emerging and Legacy Contaminants in the Foodweb in the Lower Columbia River – USGS Conhab Project** E.B. Nilsen, USGS Oregon Water Science Center / OWSC; D. Alvarez, T. Counihan, W. Cranor, J. Hardiman, J. Jenkins, M. Mesa, J. Morace, R. Patino, S. Cranor, L. Torres, I. Waite, S. Zaugg, USGS. An interdisciplinary study, USGS Columbia River Contaminants and Habitat Characterization (ConHab) project investigates transport pathways, chemical fate and effects of polybrominated diphenyl ethers (PBDEs) and other endocrine disrupting chemicals (EDCs) in aquatic media and the foodweb in the lower Columbia River. Polar organic chemical integrative samplers (POCIS) and semipermeable membrane devices (SPMDs) were co-deployed at each of 10 sites in 2008 to provide a measure of the dissolved concentrations of select PBDEs, chlorinated pesticides, and other EDCs. PBDE-47 was the most prevalent of the PBDEs detected. Numerous organochlorine pesticides, both banned and current-use, were measured at each site including hexachlorobenzene, pentachloroanisole, dichlorodiphenyltrichloroethane (DDT) and its degradates, chlorpyrifos, endosulfan, and the endosulfan degradation products. EDCs commonly detected included a series of polycyclic aromatic hydrocarbons (PAHs), fragrances (galaxolide), pesticides (chlorpyrifos and atrazine), plasticizers (phthalates), and flame retardants (phosphates). The site near Columbia City tended to have the highest concentrations of contaminants in the Lower Columbia River. In 2009 and 2010 passive samplers were deployed, resident largescale suckers (*Catostomus macrocheilus*) and surface bed sediments were collected at three of the original sites representing a gradient of exposure based on 2008 results. Brain, fillet, liver, stomach, and gonad tissues were analyzed. Chemical concentrations were highest in livers, followed by brain, stomach, gonad, and lastly, fillet. Concentrations of halogenated compounds in tissue samples ranged from 1 wet tissue. PBDEs, organochlorine pesticides, DDT and its degradates, and polychlorinated biphenyls (PCBs) were detected at all sites in nearly all organs tested. PBDE congeners most frequently detected and at the highest concentrations were PBDE-47 > PBDE-100 > PBDE-154 > PBDE-153. Concentrations in tissues and in sediments increased moving downstream from Skamania to Columbia City to Longview. Preliminary biomarker results indicate that fish at the downstream sites experience greater stress relative to the upstream site based on gonad, kidney, spleen, and liver histopathology. These results support the hypothesis that contaminant concentrations in the environment correlate to bioaccumulation in the foodweb.

**129 Analytical Considerations for the Determination of Petroleum Biomarkers** M.J. Benotti, Battelle / Analytical and Environmental Chemistry; R. Lizotte, K. McInerney, J. Thorn, Battelle. Petroleum biomarkers are a structurally diverse class of hydrocarbons found crude oils representing diagenetic transformation products of biomolecules. The relative distribution of individual biomarkers can provide information about the source or formation conditions of a particular oil and can be used to fingerprint the



material in environmental forensic applications. This talk will include 1) a summary of the analytical considerations necessary to maximize analytical sensitivity; 2) an illustration of the approach to chemical fingerprinting and source or formation conditions; and 3) a discussion of the future of petroleum biomarker analyses. Gas chromatography mass spectrometry (GC-MS) techniques have been used for several decades to measure concentrations of biomarkers in oil and sediment. However, because petroleum biomarkers are present in relatively low concentrations (compared to other classes of hydrocarbons like saturated hydrocarbons or polycyclic aromatic hydrocarbons), increasing the sensitivity of the GC-MS may require silica-gel cleanup to remove the aromatic fraction or molecular sieve cleanup to remove n-alkanes. Using a large dataset of crude oils and refined products, examples of chemical fingerprinting and the determination of source or formation conditions will be presented. Examples will include information that can be determined from individual compounds and common diagnostic ratios. Lastly, the talk will explore the future of petroleum biomarker analyses, including the potential applications for different types of detectors such as GC tandem mass spectrometry (GC-MS/MS), GC quadrupole time-of-flight mass spectrometry (GC-QToF-MS) and comprehensive GC time-of-flight mass spectrometry (GC×GC-ToF-MS).

### 130 CERCLA Well 12A, Tacoma, WA Contaminated Soil Excavation

**Case Study** R.A. Weiss, Seattle District US Army Corps of Engineers / Environmental Engineering and Technology Section; M. Michalsen, U.S. Army Corps of Engineers / Environmental Engineering & Technology Section; K. Kunas, M. Scarsella, Seattle District US Army Corps of Engineers; A. Franz, D. Giardrone, CDM Smith; M. Roberts, J. Harris, KEMRON Environmental Services, Inc.. The Well 12A Superfund Site in Tacoma, Washington is a former oil recycling facility where off-site migration of chlorinated solvent-contaminated groundwater has impacted the City of Tacoma Well 12A drinking water well. Additional site soil and groundwater contaminants include polychlorinated biphenyls (PCBs), other volatile organic compounds (VOCs), metals and dense non aqueous phase liquid (DNAPL). Reduced pump and treat system effectiveness resulted in a 2009 Record of Decision (ROD) Amendment, which specified a combination of soil excavation, in situ thermal remediation and enhanced anaerobic bioremediation to reduce groundwater contaminant mass leaving the site by 90%. The excavation component of the remedial action included removal of highly contaminated "filtercake" fill material, a 14,000 gallon underground storage tank, and related tank waste material. Two 50' x 50' excavation areas were selected from numerous sampling investigations used to model contamination greater than 1000 µg/kg VOCs in shallow soil (0-5 ft bellow ground surface [bgs]). Given the well characterized nature and small excavation area, direct dig and haul disposal was planned to accelerate the excavation process. However, the excavation complexity and project schedule were impacted from elevated soil contamination levels encountered (>3,000 µg/kg VOCs) and oily soil extending to 15 ft bgs in the direction of an on-site building which required geotechnical stabilization. On-site treatment using lime-activated persulfate was implemented to meet LDR disposal requirements and allowed landfill disposal versus incineration. On-site treatment ultimately decreased disposal travel distance by 1260 miles per trip and decreased the total excavation cost by ~ \$1,000,000. This case study will present lessons learned and information on how the conceptual site model developed from numerous extensive sampling events still allowed for major discrepancies in the overall magnitude and extent of site contamination. In addition, sampling methodologies used during excavation to characterize on-site treatment will be described along with sampling techniques used to characterize waste for final disposal. Excavation activities were completed by KEMRON Environmental Services, Inc. (KEMRON) on contract to US-ACE Seattle District, who was tasked with implementing the three remedial action components by EPA Region 10 through an Interagency Agreement.

### 131 Improved Separation of Complex Polycyclic Aromatic Hydrocarbon Mixtures Using Novel Column Combinations in GC×GC/ToF-MS

C. Manzano, Oregon State University / Department of Chemistry; E. Hoh, San Diego State University / Graduate School of Public Health; S. Simonich, Oregon State University / Depts. of Chemistry and Environmental & Molecular Toxicology. Complex mixtures of polycyclic aromatic hydrocarbons (PAHs) are difficult to resolve because of the high degree of overlap in compound vapor pressures, boiling points and mass spectral fragmentation patterns. The objective of this research was to improve the separation of complex PAH mixtures (97 different PAHs, including parent, alkyl-,

nitro-, oxy-, thio-, chloro-, bromo-, and high molecular weight PAHs) using GC×GC/ToF-MS by maximizing the orthogonality of different GC column combinations and improving the separation of PAHs from the sample matrix interferences, and unresolved complex mixtures (UCM) usually present in complex environmental samples. Four different combinations of non-polar, polar, liquid crystal and nano-stationary phase columns were tested. Each column combination was optimized and evaluated for orthogonality using a method based on conditional entropy that considers the quantitative peak distribution in the entire two-dimensional space. The highest chromatographic resolution, lowest synergety and highest orthogonality were achieved with column combination "C", consisting in a 10m × 0.15mm × 0.10µm LC-50 liquid crystal column in the first dimension and a 1.2m × 0.10mm × 0.10µm NSP-35 nano-stationary phase column in the second dimension. Environmental samples where then analyzed for complex mixtures of PAHs using column combination "C", including an atmospheric particulate matter with diameter < 2.5µm (PM<sub>2.5</sub>) sample from Beijing, China, a soil sample from St. Maries Creosote Superfund Site, and a sediment sample from the Portland Harbor Superfund Site. Column combination "C" resulted in low interference from UCM and thus better chromatographic separation and identification of PAHs. In addition, the use of this column combination in GC×GC/ToF-MS resulted in significantly shorter analysis times (176 min) for complex PAH mixtures compared to four different one-dimensional GC-methods that would be needed in order to complete the analysis of a complex PAH mixture containing parent, alkyl-, nitro-, oxy-, thio-, chloro-, bromo-, and high molecular weight PAHs using a one-dimensional GC/MS, for a total run time of 257 min, as well as potentially reduced sample preparation time.

### 132 On-line SPE coupled to LC MS/MS for sensitive analysis of multiple PPCPs in Water: Benefits versus off-line extraction and Large Volume Injection (LVI)

T. Anumol, S. Merel, S. Snyder, University of Arizona / Chemical & Environmental Engineering. With the development of advanced technologies for the treatment of Trace Organic Contaminants (TrOCs) and the synergistic effects of low doses of these compounds presently unknown, the need for analytical measurement of these compounds at very low levels in water and sewage has become paramount. The traditional method to achieve sensitivity and low levels of measurement has been to use off-line solid phase extraction (SPE) either manually or by using an automated apparatus. However, this method requires a large sample volume, is time-consuming and labor intensive. Trace enrichment or on-line SPE eliminates all these difficulties while providing sample clean-up and reduces interferences which large volume injection (LVI) cannot do. In this study, A trace enrichment cartridge (on-line) setup is compared to off-line spe for sensitivity. A single method for the analysis of over 20 pharmaceuticals and personal care products (PPCPs) using on-line spe coupled to an Agilent 6460 LC MS/MS will be shown and the results will be compared against off-line spe across different water matrixes. Critical parameters like sample volume, life of cartridge and elution solvents will also be discussed. The results of this study indicate that online spe when performed correctly offers significant benefits in time and cost while also offering extremely good sensitivity for low level TrOC detection in water.

### 133 Pyrethroid Analytical Methods for Wastewater Monitoring: An Intercomparison Exercise to Determine Suitability of Methods and Reporting Limits

J.C. Markle, Coalition for Urban/Rural Environmental Stewardship; B.H. van Buuren, Van Buuren Consulting, LLC; A.C. Barefoot, Du Pont Crop Protection / Crop Protection Products. Pesticides containing pyrethroid active ingredients have become a highly requested parameter group for low-level chemical analysis in water monitoring programs. This poses a problem to project managers, as there are no standardized methods or commercially available certified reference materials for these analyses. In addition, laboratories may vary in their instrumentation, levels of sensitivity, and techniques for extraction, clean-up, and analysis. In response to the 2006 California DPR data re-evaluation, the Pyrethroid Working Group and Tri-TAC, representing Publicly Owned Treatment Works (POTWs), developed a monitoring project for eight pyrethroids in influent, effluent and biosolids. During the project design phase, an intercomparison exercise was conducted to test the comparability of pyrethroid results from several laboratories, identify areas for method modification, and establish project reporting limits. The intercomparison exercise used real-world matrices and a process that would replicate the monitoring project's design. Two POTWs supplied samples to three participating laboratories. Each

laboratory then ran three analytical replicates of each sample in conjunction with their standard quality control samples. This generated 144 results for each of the effluent, influent, and biosolids matrices – a total of 432 data points. Laboratory reporting limits ranged from 0.5 ng/L in the effluent to 182 ng/g in the biosolids. Based on the relative standard deviations of these results, project management identified ten potential improvements to the study design – including method modifications for the biosolids matrix, and laboratory use of a stable isotope internal standards. The intercomparison allowed project managers to optimize methods, reporting limits, quality control requirements, and logistics prior to sample collection. Limitations were addressed preventatively, rather than via costly resampling and reanalysis. Intercomparisons are a useful and cost-effective tool when applied to parameter groups that are not supported through standardized methods and certified reference materials.

**134 Spatial and Temporal Mapping of Organic Contaminants Using Equilibrium Passive Samplers** P. Vlahos, University of Connecticut / Departments of Marine Sciences and Chemistry; D. Cady, Y. Son, University of Connecticut. One of the greatest challenges in the study of the fate and transport of organic contaminants is the need for long term, synoptic data. Here we present a spatial and temporally resolved time series for Long Island Sound's coastal marine waters based on 8 stations over July 2011 to July 2012. The time series uses ethylene vinyl acetate based, thin film equilibrium passive samplers that yield a quantitative measure of organic compound concentrations in water over a wide range of solubilities (Log Kow between 1.5 to 8). Samplers were deployed in triplicate for at least two weeks at each station and replaced upon retrieval. Samplers were then mailed or delivered back to the University of Connecticut where they were solvent extracted and analyzed by GCMS. Initial time series focused on polycyclic aromatic hydrocarbons (PAHs) and currently used pesticides though extracts were also archived for additional compound classes including polybrominated diphenyl ethers and phthalates. Precision at each deployment was within 20% and detection limits were in the ng/L to pg/L range for the current configuration though this may be improved by increasing the sampler surface area. Results have been embedded into Google Earth in order to illustrate the spatial and temporal advantages of this approach.

**135 An Incremental Sampling Methodology (ISM) Approach for the Characterization of Intertidal Sediment for Use in a Human Health Risk Assessment** S. McGroddy, B. Bergquist, Windward Environmental LLC. Incremental Sampling Methodology (ISM) is a structured composite sampling and processing protocol that was developed for the characterization of soil, in which samples from numerous increments (i.e., incremental samples) within areas designated as decision units are collected, combined, and analyzed in order to provide a robust estimate of the mean contaminant concentrations within the decision unit. The use of this methodology requires the identification of decision units consistent with the site conceptual site model and a protocol for deriving appropriate statistical values from the data prior to data collection. We present the use of ISM as a case study to characterize the intertidal sediment of the East Waterway in Seattle, Washington for use in the assessment of risk to humans from direct contact with sediment as a result of clamming and habitat restoration. The decision unit for human exposures was the intertidal area throughout the entire waterway. In order to develop exposure metrics such as 95<sup>th</sup> upper confidence limits on mean values, 3 replicate ISM samples were created from 96 incremental samples were collected from throughout the East Waterway, with 32 unique incremental samples for each replicate ISM sample. For most of the contaminants of concern, the variance among the three replicates was low. However, the results for polycyclic aromatic hydrocarbons (PAHs) had a high variance. Within the single exposure unit, 11 intertidal areas were created from which archived incremental samples were used to create composites for each area, which provided additional spatial information for the purpose of developing remedial alternatives. Advantages of this methodology for sampling intertidal sediment are the representativeness of mean concentrations because they are based on contributions from a large number of locations and the reduced analytical costs. Disadvantages include limitations in spatial distribution information within the decision units, and relatively increased field costs. Collecting replicate incremental samples and archiving them can enable further analyses to provide additional spatial distribution for contaminants of particular concern.

**136 Community engagement in sampling: benefits and tradeoffs using passive samplers deployed by volunteers to survey organic pollutants across an estuary** V.P. Sacks, ENVIRON / Graduate School of Oceanography, University of Rhode Island; R. Lohmann, Graduate School of Oceanography, University of Rhode Island. In recent years, passive samplers have made sampling hydrophobic organic contaminants in water and porewater easy and reliable. An advantage to using passive samplers is a comparative decrease in sample contamination issues typically associated with traditional “active” water sampling. Moreover, passive samplers are particularly easy to deploy, and persons unfamiliar can be appropriately trained on their deployment and retrieval. In this study, a group of citizen volunteers deployed passive samplers to survey organic contaminant concentrations. As part of a larger project, this element of the study helped to educate the community on the concepts of organic pollution and involve them in the science of their environment. A workshop was held to educate our volunteers and distribute sampling materials (polyethylene passive samplers, gloves, etc.). Volunteers each deployed a sampler near their homes across the Narragansett Bay (NB) watershed, thereby greatly expanding the geographic scope of our study. Of 32 samplers distributed, 25 were recovered and analyzed for the emerging contaminants alkylphenols (APs), triclosans, polybrominated diphenyl ethers (PBDEs), and legacy pesticides (OCPs). Concentrations of APs were found at about 2-3 orders of magnitude lower than their respective EC<sub>50</sub> values. Triclosan, which was undetected in NB water and porewater in a concurrent study, was detected in the volunteer sampling locations upriver near Providence, RI. Methyl triclosan, a derivative was consistently detected throughout the watershed. PBDEs 28, 47, 49, 99, and 100 were consistently found in the hundreds of fg L<sup>-1</sup> range, and PBDEs 8, 15, 153, and 154 were detected at similar concentrations in a few samples. Nine OCPs were detected in >85% of the samplers analyzed. In this presentation, we will discuss the tradeoffs and benefits of working with volunteers in this sampling effort, discuss advantages of this type of sampler, and present spatial concentrations of a variety of chemicals. Data from our volunteer deployment are compared to data from a deployment by trained scientists involved in the larger study. This study focused on involving the community to collect data for our study. By doing so, we provided community involvement, obtained useful data, and provided lower-cost sampling over a wider area than otherwise possible. We will present data quality considerations and lessons learned in this talk.

**137 Pathway-Based Analysis of Fish Transcriptomics Data Along Effluent Gradients in Minnesota Rivers** J. Berninger, Baylor University / Department of Environmental Science; D. Martinovic, University of Saint Thomas, University of St. Thomas / Dept. of Biology, U.S. EPA, Mid-Continent Ecology Division / Mid-Continent Ecology Division, University of St. Thomas / Biology, University of Saint Thomas / Biology; G.T. Ankley, US EPA / Office of Research and Development, National Health and Environmental Effects Research Laboratory, Mid-Continent Ecology Division, U.S. EPA / National Health and Environmental Effects Research Laboratory; D.L. Villeneuve, U.S. EPA / National Health and Environmental Effects Research Laboratory, U.S. EPA. As part of a larger effort to assess the health of streams and rivers influenced by municipal effluents in Minnesota, fathead minnows (*Pimephales promelas*; FHM) were exposed to ambient surface waters from three locations. The locations were generally representative of the state: urban – Rochester, agricultural – Hutchinson; rural/forested – Ely. Fish were exposed in aerated mobile exposure units supplied with water from upstream, at, or downstream of a wastewater treatment plant (WWTP) outfall. While multiple endpoints were evaluated, this study focuses on FHM female ovary transcriptomics following a 4 day exposure. Comparing between treatment groups (upstream, downstream, at effluent) within each location, the number of significantly differentially expressed genes (DEGs) were highest in Rochester (5847), than Hutchinson (1295) and Ely (867). Among the DEGs, hierarchical clustering and PCA showed distinct separation of the upstream, downstream, and effluent treatments at each location. Gene Set Enrichment Analysis (GSEA) showed the overall number of gene sets enriched followed the same pattern as the differential expression, with highest enrichment in Rochester and lowest in Ely. Comparing along the effluent gradient, different patterns were observed at each site. There were, however, a few gene sets that showed consistent responses to the effluent gradient across all three locations. Enrichment moving upstream was seen in two gene sets putatively linked with the promotion of oocyte maturation, (through gene sets associated with cyclin B and cathepsin D). Another gene set, a putative inhibitor of oocyte maturation (through a TGFB1 associated



gene set), exhibited enrichment along the downstream gradient at all locations. Given the substantial differences in land use, human population, and pollutant chemistry the consistent impact on oocyte maturation-related gene sets suggests an emerging trend across effluent gradients. In general, results from female FHM ovary transcriptomics follow a similar pattern to those of the companion analytical chemistry and studies with male FHM livers (transcriptomics and metabolomics). This study demonstrates the potential utility of transcriptomics for effects-based monitoring efforts. The contents of this abstract neither constitute nor reflect official US EPA policy.

**138 Phenanthrene induced transcriptional responses in the female fathead minnow (*Pimephales promelas*) liver** J.R. Loughery, A. Mercer, K. Kidd, C. Martyniuk, University of New Brunswick / Biology. Phenanthrene (PHEN), a polycyclic aromatic hydrocarbon (PAH), is commonly found in the aquatic environment due to natural and anthropogenic causes. PHEN has been shown to induce oxidative stress in teleosts but recent studies suggest it may also be an endocrine disrupting substance. Female fathead minnows (FHM; *Pimephales promelas*) were exposed for up to 72 hrs to waterborne PHEN at target concentrations of 0, 50 and 500 ppb (environmentally relevant) and a high concentration of 5000 ppb. Fish were sacrificed after 24, 48 and 72 hrs. Body, liver and gonad weights were recorded and livers frozen for gene expression analyses. A dose-response microarray analysis was conducted on 48 hr liver tissues on an 8x15K FHM microarray. Relative to control tissue, there were 1235, 907 and 2568 differentially regulated transcripts in 50, 500, and 5000 ppb, respectively. Of these genes, 228 were common between concentrations. A total of 1201 genes displayed significant variation when the concentrations were compared. Some of the significantly different gene transcripts were related to reproductive processes, including genes involved in zona pellucida glycoproteins (oocyte membrane proteins), and estrogen receptor binding proteins (estrogen receptor  $\alpha$  transcription activity). Gene set enrichment analysis of livers from fish exposed to 5000 ppb yielded 100 significant gene ontologies (GO). GO terms with a significant median fold change of -1.5 or less were involved in immune system function and lipid storage. GO terms with a significant median fold change of 1.5 or greater were involved in DNA replication or neuron contraction and guidance. This study demonstrates that a short-term exposure to PHEN induces transcript changes associated with reproduction and pathways associated with the immune system, DNA replication and meiosis, and lipid metabolism.

**139 Effects of cadmium and nickel on cellular antioxidants and gene transcription levels in yellow perch under chronic laboratory exposure conditions** M.A. Defo, B. Bougas, INRS / INRS-ETE; E. Normandeau, Université Laval / Biology; L. Bernatchez, Université Laval / Département de Biologie; P.G. Campbell, INRS-Eau, Terre et Environnement / INRS-ETE, INRS / Centre Eau Terre Environnement; P. Couture, INRS / Centre Eau Terre Environnement, INRS / Centre Eau, Terre et Environnement. The transcriptional response of one thousand genes to metal contamination was investigated using a cDNA microarray on adult yellow perch (*Perca flavescens*) exposed in the laboratory to cadmium (0  $\mu\text{g/L}$ , 0.8  $\mu\text{g/L}$  and 8  $\mu\text{g/L}$ ) and nickel (0  $\mu\text{g/L}$ , 60  $\mu\text{g/L}$  and 600  $\mu\text{g/L}$ ) for 6 weeks. At the end of exposure, although kidney cadmium and nickel concentrations were significantly different among the exposed groups, differences in transcription level were detected only at the highest concentration, where a total of 106 genes were differently expressed for both metals. Among these, 2 genes involved in cell oxidative stress were of particular interest: the transcription level of catalase (*cat*) was increased whereas that of microsomal glutathione S-transferase 1 (*mgst1*) decreased with the level of metal contamination. We propose that the transcription level of oxidative stress-related genes can be a good indicator of cadmium and nickel exposure. Coupled with field validation, these findings will orient future physiological and biochemical studies aimed at identifying mechanisms of metal toxicity in fish under chronic exposure conditions.

**140 Genetic pathways underlying the immediate and lifespan impacts of a developmental exposure to the endocrine disrupting herbicide atrazine** G. Weber, Purdue University / School of Health Sciences; S. Peterson, S. Lewis, Purdue University; T.L. Lin, Purdue University / Comparative Pathobiology; M. Sepulveda, Purdue University / Forestry and Natural Resources; J. Freeman, Purdue University / School of Health Sciences. There is a growing body of evidence which indicates continual exposure to endocrine disruptors over the lifespan of an individual or during increased

sensitivity windows (such as development) substantially elevates the risk to develop multiple types of diseases. Atrazine, an herbicide commonly applied to agricultural areas throughout the Midwest and a common contaminant of potable water supplies, is implicated as an endocrine disruptor and potential carcinogen. The specific adverse health effects associated with atrazine exposure and the underlying molecular mechanisms of these effects are not well defined. In an effort to delineate the mechanisms of atrazine toxicity, we exposed zebrafish embryos to environmentally relevant concentrations of atrazine shortly after fertilization through 72 hours post fertilization (hpf). Transcriptomic profiles immediately following the embryonic atrazine exposure were obtained and subsequent analyses identified an enrichment of genes with altered expression patterns that are involved in neuroendocrine development and function, cell cycle regulation and carcinogenesis. A subset of individuals was permitted to mature under normal conditions to evaluate the persistent effects of developmental exposure to atrazine in these adults and in unexposed subsequent generations. A significant difference in the number of pairs that successfully bred in one of the atrazine treatment groups coupled with a distinct altered reproductive morphological phenotype in ~10% of female adult zebrafish was observed indicating a persistent lifespan effect of the developmental atrazine exposure. These females were substantially larger in size, particularly in the area of the ovaries. Furthermore, gene ontology analysis from microarrays performed on adult female zebrafish brains that were developmentally exposed showed enrichment for genes involved in neuroendocrine system function and carcinogenesis. The reproductive alterations observed in a subset of females along with decreased mating success and altered transcriptomic profiles provide support to the endocrine disrupting effects of this herbicide and warrant further investigation. Current efforts are aimed at assessing the transgenerational effects of this developmental atrazine exposure in subsequent unexposed generations by focusing on epigenetic regulatory processes and targets that may be altered in response to atrazine exposure.

**141 Effects of Disperse Yellow 7 in *Silurana tropicalis* Gene Network Analysis** V.S. Langlois, Royal Military College of Canada / Chemistry & Chemical Engineering; J. Mathieu-Denoncourt, Royal Military College of Canada; C.J. Martyniuk, University of New Brunswick / Dept. of Biology/ Canadian Rivers Institute, University of New Brunswick / Biology; S.R. de Solla, Environment Canada / Science and Technology Branch. Dyes used in fabric and leather industry are being released into Canadian ecosystems. Recent studies have demonstrated that dyes made of azo compounds are toxic to biota, which is explained by their toxic metabolites (e.g., aromatic amines) produced. The metabolites of azo compounds interact with hydrophobic surfaces of cell membranes and expand them which impede normal cellular functions. It has been suggested that this process leads to cell death due to improper ion balance. Currently, it is estimated that between 10 and 15% of azo dyes are released in the environment as effluent. A recent study conducted by our laboratory has demonstrated that the azo dye Disperse Yellow 7 (DY7) induced cellular stress and interfered with androgen biosynthesis in early tadpole development. The aim of this study was to evaluate gene networks altered by azo compounds in amphibians using ecotoxicogenomic approaches. Larvae of the frog *Silurana tropicalis* (Western clawed frog) were exposed to sediment spiked with up to 887 ppm DY7. Larvae were exposed from Nieuwkoop-Faber developmental stage 12 to 46. At exposure completion, RNA was isolated from whole larvae and quality was ascertained using bioanalyzer analysis. Our custom Agilent 4 X 44 K microarray for *S. tropicalis* was used to investigate gene regulatory networks. Four control and four treated biological samples were investigated. Following exposures, heat shock protein (hsp) 70 and 90 mRNA levels increased by 2.5 and 2.4 fold, respectively, suggesting a response to cellular stress, DY7 also affected the androgen-related genes *ar* and *srd5a2*. This study presents the transcriptional regulatory pathways affected by DY7 in *S. tropicalis* early development.

**142 Effect of Glyphosate on the Genomic DNA of African Snail, *Archachatina marginata*** A.C. Udebuani, Federal University of Technology / Dept. of Biotechnology; I.N. Onwurah, University of Nigeria / Dept of Biochemistry, University of Nigeria, Nsukka / Department of Biochemistry, University of Nigeria / Biochemistry, University of Nigeria. The study of the effects of contaminants of emerging concern such as glyphosate on non-target organisms such as African snail (*Archachatina marginata*), with emphasis on the genomic DNA profile, is of great toxicological importance in terms of risk assessment. Until relatively recently, genetic effects were



largely inferred from observations of genotype. With the ability to perform DNA sequencing and use of PCR, it has become possible to determine genetic variations occasioned by environmental toxicants through the science of Genomics. *Archachatina marginata's* nature and way of life offers it an excellent model animal for the study of the sensitivity of some terrestrial animals to environmental stress induced by chemical pesticides such as glyphosate. In this study, the African giant snail was exposed to soil samples spiked with different concentrations of glyphosate for several weeks and their genomic DNA extracted and subjected to electrophoresis. Amplification of the various DNA bands was carried out using an OMnE-PCR System programming. Our result shows that the AP-PCR genotypic profile with oligonucleotide prima OPA (gaaacgggtg) on DNA of *Archachatina marginata* exposed to glyphosate for a period of 7 days gave varying number of fragments with different amplification bands, corresponding to different molecular weights or sizes. Similarly in comparing the length of time of exposure to glyphosate, it was observed that newer DNA fragments with higher intensity and larger molecular sizes were obtained from DNA amplification as against the control that remained the same, except for the unique band (100-400bp) that was observed in all the snails (exposed and unexposed). It can be inferred from this study that since glyphosate caused increased number of DNA fragments and significantly varying molecular sizes in the African giant snail after an exposure, it has the potential mutagenicity that could induce genotoxicity on non target organisms.

**143 Looking for biomarkers of Hg exposure by transcriptome analysis in the aquatic plant *Elodea nuttallii*** N. Regier, University of Geneva; C. Cosio, Geneva University, Geneva University / Forel Institute. To gain insight into the impact of metal contamination on ecosystems, the potential of various tests on metal accumulating organisms is explored. Rooted macrophytes are well-suited to be used in ecotoxicological tests because they are exposed to both water and sediments. *Elodea nuttallii* is a rooted macrophyte native to Northern America, but as a neophyte, has spread throughout the world. It is able to accumulate large amounts of metals, including Hg and Cd, and is therefore a good candidate for the aim of this study. In the present work we used transcriptome analysis (RNA-seq) of *Elodea nuttallii* to identify biomarkers of Hg exposure. RNA-seq yielded a total number of 63'596 contigs. A total of 27'669 sequences could be assigned to at least one GO term. We also used the functional catalogue of MIPS to identify which biological functions were affected by Hg and Cd exposure. In brief, these results can be interpreted such that *E. nuttallii* responded to the treatments by a change in energy metabolism caused by the inhibition of photosynthesis, and an adaptation of homeostasis networks to control accumulation of Hg. We identified transcripts regulated in a dose-dependent manner in response to Hg and Cd. By applying these criteria, we found a total number of 212 differentially expressed genes in response to Cd, and 170 genes in response to Hg. We further performed a global expression analysis using the Nanostring nCounter for this subset of genes to evaluate whether gene expression observed in metal-specific treatments was able to predict metal exposure in a complex environment. We found two main clusters: samples exposed to high ( $\mu\text{g/L}$  range) and low ( $\text{ng/L}$  range) metal concentrations, suggesting that more genes would be needed to discriminate between the different metals. Samples exposed in the field and to natural contaminated sediments clustered well with samples exposed to low metal concentrations under laboratory conditions. Plants exposed to darkness, cold or salinity also showed a response, although different from the metal exposed samples. Genes identified in the laboratory after exposure to high concentrations were successfully used as biomarkers of Hg exposure in the field in a complex environment and resulted more sensitive than other endpoints. Data suggest that a genomic approach could be used to develop water and sediment quality tests with unsequenced organisms and confirmed the interest of rooted macrophytes for this aim.

**144 Whole genome data and its potential use in the omics-based researches of the self-fertilizing fish, *Kryptolebias marmoratus*** J. Lee, Hanyang University / Dept of Chemistry, Hanyang University Graduate School / Dept of Chemistry; J. Rhee, Hanyang University. Genome resources of certain organisms may offer several advantages for a better understanding of diverse areas of biological patterns and functioning. The omics platform makes useful approaches possible for the study of organs and entire organisms. These approaches can be applied as powerful screening tools for whole genome, proteome, and metabolome profiling, and can also be utilized to understand molecular changes in response to internal and/or

external stimuli. This methodology has been already successfully applied to the model fish species such as zebrafish and medaka in diverse research areas such as basic physiology, developmental biology, genetics, and environmental biology. To develop *K. marmoratus* as another model species that has unique characters and research merits, we obtained the whole genome data from *K. marmoratus*, and address here how we can use such data to obtain genome resource-based molecular mechanistic findings. Here, we review the current developmental status of genome information of *K. marmoratus* to provide a start line for omics approaches. We evaluate the potentials and applications of integrated-omics platforms for future studies, particularly in environmental science, developmental biology, and cancer science. We conclude that *K. marmoratus* whole genome information will allow us to better understand molecular functions of genes, proteins, and metabolites that are involved in diverse biological functions in this species. Each omics platform and particularly combined technologies making effective use of bioinformatics will provide a powerful tool for hypothesis-driven investigations and discovery-driven discussions on diverse aspects of this species and probably of fish and vertebrates in general.

**145 A Mesoscale Total Dissolved Solids Quantity and Quality Study Integrating Responses of Multiple Biological Components in Small Stream Communities** C. Nietch, U.S. EPA, Office of Research and Development, National Risk Management Laboratory / Water Supply Water Resources Division, U.S. Environmental Protection Agency / Water Supply Water Resources Division, U.S. EPA / Water Supply Water Resources Div; J. Lazorchak, United States Environmental Protection Agency / Ecological Exposure Research Division; B. Johnson, U.S. EPA / Ecosystems Research Branch; D. Brown, U.S. EPA / Water Supply and Water Resources Division; B. Ramakrishnan, Shaw Environmental Inc. / EPA Experimental Stream Facility; H. Rogers, Student Services Contractor; P. Weaver, The McConnell Group, c/o U.S. EPA; S. Decelles, The McConnell Group c/o U.S. EPA; D. Macke, US EPA / ORD; K. Daniels, US EPA / Water Supply and Water Resources Div; J. Allen, U.S. EPA / Office of Research and Development, U.S. EPA / ORD; K. Patnode, USFWS / Pennsylvania Field Office. A 42-day dosing test with ions comprising an excess TDS was run using mesocosms colonized with natural stream water fed continuously. In gridded gravel beds biota from microbes through macroinvertebrates are measured and interact in a manner realistic of stream riffle/run ecology. Single species assays were run in-parallel using the equivalent mesocosm matrices. Although the species assemblage and background water chemistries of the mesocosms may not translate to certain field locations, the intent was to study the potential change in the ecologies of community interactions. Paired with the single-species assays the approach provides community context to standard toxicological tests. The excess TDS was comprised of  $\text{Cl}^-$ ,  $\text{Na}^+$ , and  $\text{Ca}^{2+}$ , which are the primary ions making-up produced waters of Central Appalachian shale brines. The realized TDS concentrations were 247, 370, 590, 742, 1288, 2066, 3615, and 6963 ( $\text{mg/L}$ ). Targets were based on mixing-models from fifty Pennsylvania oil and gas wastewater permits and estimated low flows of their receiving streams. An increasing trend in periphytic algal biomass happened across doses higher than 590  $\text{mg/L}$ . Visible differences in algal community structure occurred after 742  $\text{mg/L}$  including a shift to a high percentage of red alga (*Compsopogon*) coverage, increased density of long-chain diatom filaments, and a decrease in cyanobacteria colonies. These changes coincided with community changes that were significant after the 1288  $\text{mg/L}$  dose for macroinvertebrates colonizing the mesocosms. Chironomids and Elmidae were the primary larval insects. They responded oppositely to the excess TDS with a respective increase and decrease in doses higher than 2066  $\text{mg/L}$ . EPT taxa (only 3% of individuals) generally decreased with increasing TDS. Polycentropid caddisfly nets were absent in the two highest doses. Worms and crustaceans exhibited negative dose-responses, while snails appeared unaffected. Interpreting the single-species exposures were more straightforward. They suggested significant (LC50) lethality near the 2066  $\text{mg/L}$  TDS dose for two species of mussels and daphnids, while a mayfly exhibited higher sensitivity. All endpoints considered, the data suggested that a chronic dose of greater than 590  $\text{mg/L}$  could start to alter the producers in streams, while varied responses in the consumers have significant community-wide consequence at exposures greater than 1288  $\text{mg/L}$  and when the TDS is dominated by  $\text{Cl}^-$ ,  $\text{Na}^+$ , and  $\text{Ca}^{2+}$  ions.

**146 An Overview on EPA's Research on Potential Impacts from Hydraulic Fracturing on Drinking Water Resources** C.A. Impellitteri, US EPA / Water Supply and Water Resources Division, US EPA / Environmental

Engineer; J. Briskin, U.S. EPA OAA / OSP. The United States Environmental Protection agency has undertaken research to examine the potential effects of hydraulic fracturing (HF) on drinking water resources. The research encompasses the total water cycle as it is used in hydraulic fracturing; from water procurement for HF operations to water treatment and disposal. The research will also provide information on potential impacts from HF operations on groundwater and surface water sources. This presentation will provide a basic overview on water issues related to hydraulic fracturing in the United States. The presentation will also summarize EPA's research plan and an update of progress on efforts such as: field case studies, water consumption, water quality monitoring and water/wastewater treatment research. A summary of the current cross-agency plans for future environmental HF research will also be presented.

**147 Aquatic macroinvertebrate communities across a gradient of natural gas activity in streams** S. Entekin, University of Central Arkansas / Biology; N. Jensen, A. Musto, J. Kelso, University of Central Arkansas; B. Austin, M. Evans-White, University of Arkansas; C. Gallipeau, The Nature Conservancy; B. Haggard, University of Arkansas; E. Inlander, The Nature Conservancy; L. Massey, University of Arkansas. Advances in drilling and extraction of natural gas have resulted in rapid expansion of wells and associated infrastructure in shale basins across the US. Rate of gas well installation in the Fayetteville Shale of north-central Arkansas has been 774 wells per year since 2005 with more planned. Rapid and concentrated activity and wells placed close to streams increases potential for negative effects to aquatic biological communities. We quantified a suite of water quality metrics at storm and base flow including trace element concentrations, conductivity, and suspended sediments from autumn 2009 to autumn 2011. Macroinvertebrates were quantified across the same study sites in spring 2010 and spring 2011. We predicted higher concentrations of suspended sediment, conductivity, and trace elements in catchments with more gas well activity and a concurrent decline in macroinvertebrate density, diversity, and some species traits. Pearson correlations were used to explore relationships among gas activity, sediment and trace elements, and macroinvertebrates across the gradient of gas activity represented by the study sites. Turbidity during storms was positively related to gas well activity during six of seven storms, organic sediment was positively related to gas activity in two of seven storms, and inorganic sediment was positively related to gas activity in four of seven storms. In 2010, macroinvertebrate density was positively related to gas activity ( $r=0.85$ ,  $p=0.002$ ) and diversity was negatively related to suspended inorganic sediment concentration ( $r=-0.98$ ,  $p<0.001$ ). In 2011, most trace elements (e.g., Al, Pb, Ni) and conductivity were positively related to gas well densities ( $r>0.6$ ,  $p<0.05$ ). Multivoltine macroinvertebrate taxa were positively related to inorganic sediment concentrations ( $p=0.007$ ,  $r=0.71$ ) and diversity declined with fine organic matter ( $p=0.02$ ,  $r=-0.68$ ). Few community-level metrics were related to trace element concentrations, although two mayfly taxa (*Caenis* and *Baetis*) tolerant to sedimentation were strongly positively related to gas well density and an increase in nickel and lead concentrations ( $p<0.05$ ,  $r>0.7$ ).

**148 Impact of natural gas wells on periphyton and metabolism in streams in north central Arkansas** B. Austin, University of Arkansas Fayetteville / Department of Biological Sciences; K. Brick, University of Arkansas Fayetteville; M. Evans-White, University of Arkansas Fayetteville / Department of Biological Sciences; S. Entekin, University of Central Arkansas / Biology; E. Inlander, C. Gallipeau, The Nature Conservancy. Recovery of natural gas has increased significantly in recent years. Construction of natural gas wells (NGWs) increases the potential for sediment erosion into streams, which could negatively influence periphyton communities. The goal of this study was to determine if increasing NGWs within catchments negatively impact stream periphyton biomass and metabolism. Ten streams with varying catchment densities of NGWs (0-3.2 wells/km<sup>2</sup>) were sampled during winter and spring of 2010 and 2011 to examine the effects of NGWs on periphyton and metabolism. Two cobbles from three riffles and three pools were collected within each stream to quantify periphyton biomass in the form of chlorophyll *a* (chl *a*) and ash-free dry mass (AFDM). Whole stream metabolism was estimated from diel curves of dissolved oxygen at a single station, within each stream over a three day period. In addition, dissolved nutrients, turbidity, and light transmittance were measured across sites and land use variables were quantified for each catchment using ArcMap. We used multiple linear regressions to examine effects of NGWs, well density, land use, and water quality on periphyton

and metabolism response variables. Chlorophyll *a* was positively related to the number of wells in a catchment during both winter sampling events (2010  $r^2=0.79$ ,  $p<0.01$ ; 2011  $r^2=0.57$ ,  $p=0.01$ ). Gross primary production (GPP) was positively related to well density during spring 2010 and winter 2011 sample periods ( $r^2=0.46$ ,  $p=0.05$ ;  $r^2=0.83$ ,  $p=0.01$  respectively). Nutrients and light transmittance were not positively related to chlorophyll *a* or GPP. Therefore, NGW effects on additional environmental factors, such as on densities or biomass of grazing macroinvertebrates, might explain the positive effect of NGWs on stream periphyton and GPP. Our findings suggest that increases in primary production may result from NGW development in catchments. Periphyton and primary production metrics may be useful indicators of NGW impacts on streams.

**149 Marine shales are a source of selenium to aquatic and terrestrial food webs in a watershed being developed for energy** T.S. Schmidt, US Geological Survey / Water Resources Div, USGS / Water Resources Div; R.E. Wolf, U.S. Geological Survey; C.A. Stricker, C.R. Bern, M.L. Clark, J.M. Holloway, R.R. McDougal, USGS. Some energy development in Muddy Creek, WY, occurs on marine shales. These shales are natural sources of selenium (Se) that, when disturbed, can mobilize Se into streams. We assessed Se dynamics in soil, sediment, water, and aquatic (damselfly larvae) and riparian (spiders and adult damselflies) food webs at 9 sites to develop baselines and assess risks to wildlife prior to expansion of oil and gas development. Different shale formations contribute varied Se concentrations to stream sediment and water. Sediments control the concentration of Se in dissolved and particulate phases and partitioning of Se from dissolved to particulate phases was exceptional ( $k_d = 3000$ ). Sediment controls on Se bio-availability to the aquatic and terrestrial food web were observed via strong negative relations between Se concentrations in damselfly (Lestidae and Coenagrionidae,  $r^2=0.87$ ) larvae and spiders (*Tegrynatha*,  $r^2=0.68$ ). Adult damselfly Se concentrations showed no trend with Se in larvae, soil, water, or sediment. Se concentrations in emergent aquatic insects were higher than those in terrestrial arthropods suggesting that spider exposure to Se was via aquatic prey. Ecosystem-scale modeling of Se exposure demonstrated substantial risks to aquatic and terrestrial wildlife via aquatic sources and potentially even greater risks to birds if emergent aquatic insects and spiders were considered as prey items. We conclude damselfly larvae and riparian spiders are good indicators of aquatic and terrestrial exposure, respectively, to Se derived from marine shales and that risks to terrestrial wildlife might be underestimated if pathways of exposure that link aquatic and terrestrial food webs are not considered.

**150 Relationship Between Mountaintop Mining Activities and Macroinvertebrate Communities in Streams in Southwestern West Virginia** S.P. Canton, GEI Consultants, Inc. / Ecological Division; N. Burbank, G. DeJong, J. Lynch, GEI Consultants, Inc.. Recently, the relationship between mountaintop removal coal mining/valley fill (CM/VF) activities and macroinvertebrate communities in Appalachian streams has been considered. CM/VF is thought to elevate concentrations and levels of certain stressors, including sulfate and conductivity, which is in turn thought to negatively affect macroinvertebrate communities in streams that drain CM/VF operations. The purpose of this study was to evaluate the ecological impacts of CM/VF activities on macroinvertebrate communities in streams draining valley fills. We sampled the benthic macroinvertebrate populations at multiple sites in 12 streams in southwestern West Virginia, including 9 streams that drain valley fills and 3 reference streams with no upstream mining. We analyzed the relationship between certain macroinvertebrate community metrics, including number of taxa; number of Ephemeroptera, Plecoptera, and Trichoptera taxa; percent Ephemeroptera individuals; and West Virginia Stream Condition Index (WVSCI) score, and measures of watershed disturbance, such as mining influence (e.g., percent of upstream area mined), conductivity, and sulfate concentrations. We found that the macroinvertebrate community metrics generally indicated less ideal conditions with increasing amounts of mining, conductivity, and sulfate, but these trends were not always strong or statistically significant. Previous analyses of these data indicated that a combination of ionic chemistry, soil characteristics, substrate characteristics, and channel features related to erosion were influential in determining the structure of macroinvertebrate communities. Given the results of the current and prior studies, the effect of CM/VF activities on macroinvertebrate communities appears to be related to a complex combination of multiple factors that may be related to watershed disturbance, changes in water chemistry, and variation in habitat quality.

**151 The Dangers of “Proofiness” in the Evaluation of Mountaintop Removal Coal Mining Impacts** C. Stahl, U.S. EPA Region III, U.S. Environmental Protection Agency, Region III / Environmental Assessment and Innovation Division, U.S. EPA Region III / Environmental Assessment and Innovation Division, U.S. EPA Region III / Air Protection Division; A. Cimorelli, U.S. Environmental Protection Agency, Region III; J. Babendreier, U.S. Environmental Protection Agency / Office of Research and Development; J. Kremer, U.S. Environmental Protection Agency, Region III / Environmental Assessment and Innovation Division. Assessment of ecological and human health impacts from coal mining in West Virginia presents challenges for agencies responsible for permitting and evaluating those impacts. These challenges include correctly identifying, locating and diagnosing stressor sources and understanding the resulting impacts when observations are presented only in the integrated ecosystem. This presentation discusses the need for caution when determining the appropriateness of data and scientific tools particularly when making controversial environmental assessments. Some commonly used existing techniques and approaches evaluating the environmental and public health impacts of anthropogenic activities such as mountaintop removal coal mining will be discussed, such as the use of land cover data, regression models, sampling, monitoring and census data, and decision support tools. “Proofiness” (paraphrased from Seife’s book of the same name) is the use or misuse of mathematical arguments to arrive at misleading conclusions. Environmental assessments typically use many kinds of data and scientific tools, where the need for caution introduces uncertainty. Furthermore, combining information in these environmental assessments uses values, which introduces additional uncertainty. This presentation also discusses the need for better understanding of the role of uncertainty and values, which can be improved through the use of uncertainty analyses and decision analytic techniques. Scientists have always warned and been warned about the appropriate selection and application of techniques such as statistics and models. Scientists drawn into the high level of controversy and community urgency can fail to adequately articulate the caveats and constraints of these kinds of assessments. Therefore, additional vigilance is required when performing impact assessments, particularly when there are controversial activities being evaluated. Disclaimer: Although this work was reviewed by EPA and approved for publication, it may not necessarily reflect official Agency policy. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Environmental Protection Agency, Region III / Environmental Assessment and Innovation Division, U.S. EPA Region III / Environmental Assessment and Innovation Division, U.S. EPA Region III / Air Protection Division; A. Cimorelli, U.S. Environmental Protection Agency, Region III; J. Babendreier, U.S. Environmental Protection Agency / Office of Research and Development; J. Kremer, U.S. Environmental Protection Agency, Region III / Environmental Assessment and Innovation Division. Assessment of ecological and human health impacts from coal mining in West Virginia presents challenges for agencies responsible for permitting and evaluating those impacts. These challenges include correctly identifying, locating and diagnosing stressor sources and understanding the resulting impacts when observations are presented only in the integrated ecosystem. This presentation discusses the need for caution when determining the appropriateness of data and scientific tools particularly when making controversial environmental assessments. Some commonly used existing techniques and approaches evaluating the environmental and public health impacts of anthropogenic activities such as mountaintop removal coal mining will be discussed, such as the use of land cover data, regression models, sampling, monitoring and census data, and decision support tools. “Proofiness” (paraphrased from Seife’s book of the same name) is the use or misuse of mathematical arguments to arrive at misleading conclusions. Environmental assessments typically use many kinds of data and scientific tools, where the need for caution introduces uncertainty. Furthermore, combining information in these environmental assessments uses values, which introduces additional uncertainty. This presentation also discusses the need for better understanding of the role of uncertainty and values, which can be improved through the use of uncertainty analyses and decision analytic techniques. Scientists have always warned and been warned about the appropriate selection and application of techniques such as statistics and models. Scientists drawn into the high level of controversy and community urgency can fail to adequately articulate the caveats and constraints of these kinds of assessments. Therefore, additional vigilance is required when performing impact assessments, particularly when there are controversial activities being evaluated. Disclaimer: Although this work was reviewed by EPA and approved for publication, it may not necessarily reflect official Agency policy. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

**152 Potential Risks to Ecological Receptors Posed by Hydraulic Fracking** S. Jones, Conestoga-Rovers & Associates / Ecologist. As the practice of hydraulic fracking becomes a more widely used method for extracting natural gas from underground sources, the concerns for risk to human health and the environment are likewise increasing. To date, the vast majority of the attention from environmental and conservation groups and regulators has been directed at risk to human health due to introduction of known carcinogens into groundwater systems. Conversely, relatively little attention has been directed at potential risks to ecological receptors. Although ecological receptors are not generally not directly exposed to groundwater, exposure to chemicals and abiotic media in fracking fluids may ultimately occur through the migration and transport to streams, lakes, and wetlands. In this presentation, the framework for ecological risk assessment will be used to objectively assess the potential to risk to aquatic, semi aquatic, and terrestrial receptors posed by fracking. The assessment will identify the types of chemicals and media typically used in fracking, the mechanisms of fate and transport of fracking chemicals in groundwater, migration pathways, exposure routes, exposure points. The mechanisms of ecotoxicity and availability of ecological benchmarks for fracking chemicals will also be addressed.

**153 You Intentionally Put Pesticides in Water? The Role, Fate and Risk of Aquatic Herbicide Use** M. Blankinship, Blankinship & Associates, Inc. Currently, there are 10 herbicides registered for use in California to control aquatic vegetation. These herbicides are intentionally applied to water to control submersed, emergent, and floating weeds and algae in ponds, lakes, irrigation canals, drinking water reservoirs and flood control channels. An overview of the role, fate and human and ecological risk that the use of these herbicides pose will be presented.

**154 Occurrence of glyphosate and acidic herbicides in select urban rivers and streams in Canada, 2007** N.E. Glozier, J. Struger, Environment Canada; A. Cessna, Agriculture and Agri-Food Canada; M. Gledhill,

M. Rondeau, W. Ernst, M. Sekela, S. Cagampan, E. Sverko, C. Murphy, J. Murray, D.B. Donald, Environment Canada. Public and scientific concern has grown over the last decade in Canada over the cosmetic use of pesticides in urban centres. With this in mind a national survey was designed to monitor eight commonly used herbicides in urban rivers and streams across Canada. Samples were collected monthly on one of two pre-determined dates from April to September, 2007 from 19 sites within 16 watersheds, including 15 sites downstream of urban lands and 4 non urban sites. Additional samples were also collected approximately three times from each watershed after precipitation events. The herbicides 2,4-D, mecoprop, dicamba, glyphosate and its major metabolite AMPA were most frequently detected. Using either herbicide concentrations upstream/downstream of urban centres or bromoxynil and clopyralid as indicators of agricultural inputs of herbicides to streams, it was clear that environmental concentrations of these herbicides downstream of urban areas were linked to urban pesticide use in Canada. Herbicide concentrations were greater during or after significant rainfall events, however, none of the herbicide concentrations measured in these samples exceeded existing Canadian Water Quality Guidelines for the protection of aquatic life. This is the first time a national survey of pesticides in urban rivers has been carried out in a consistent fashion across Canada. Concentrations of 2,4-D, mecoprop, dicamba, glyphosate and AMPA were linked to urban use and frequently detected in all geographic areas. Geographical differences in concentration suggested differences in herbicide usage or stream connectivity rather than overall indicators of urban land use. Some jurisdictions in Canada have recently restricted cosmetic use of pesticides and future studies should be able to determine whether such restrictions will lead to reduced pesticide concentrations in these surface waters.

**155 Wash-off potential of a urban use herbicide from concrete surfaces: potential contributor to urban pesticide contamination** J.H. Myers,

University of Melbourne / CAPIM; V.J. Pettigrove, The University of Melbourne / CAPIM; G. Rose, P. Zhang, A. Bui, Department of Primary Industries / Future Farming Systems Research Division. Monitoring studies are showing prevalent contamination of urban waterways with pesticides, and this contamination is being linked with urban use. The finding of almost ubiquitous contamination of urban systems by herbicides in areas such as Melbourne, Australia, is of particular concern due to the acute toxicity related to these chemicals. Recent surveys by CAPIM consistently reported triazine herbicides as dominant contaminants in Melbourne’s waterways (up to 4.45µg/L). In Australia, the triazine herbicides atrazine and simazine are the second most widely used herbicides (6000 tonnes annually), with over 4000 registered uses. Impervious surfaces, such as concrete in driveways and paths are considered sources of herbicide contamination in urban areas. While transport and fate of insecticides from hard surfaces has had attention, very little is known about herbicide transport from hard surfaces. To the best of the authors’ knowledge this is the first investigation of triazine herbicide transport from hard surfaces. In the present study a simple method was used to evaluate pesticide wash-off potential from concrete surfaces. Two types of commercially available pavers, smooth and rough aggregate, were treated with an off-the-shelf product containing simazine as the active ingredient. Treatment was according to label directions. The dependence of washable simazine residues in run-off was evaluated on time exposed to outdoor conditions prior to a wash event and repeated washing events. After application to the pavers, washable fractions of simazine rapidly decreased for both paver types. Wash-off potential was greatest 1hr after pesticide application, with simazine concentrations in run-off reduced by ½ that of 1hr concentrations after 2d. These concentrations remained stable for repeated washings up to 90d (continuing) and for exposure to outdoor conditions of 14d prior to a wash event. Smooth pavers showed a high concentration of simazine to be washed off during the first washing (1330µg/L), compared to that off rough aggregate (835µg/L). As time exposed to outdoor conditions prior to washing increased, or with repeated washings, simazine concentrations in run-off were higher for rough aggregate pavers (average 485µg/L compared to 420µg/L). Results from this study indicate that simazine residues remain on concrete and are available for contaminating runoff for months after application.

**156 Importance of loose particles in pesticide runoff from urban concrete surfaces** J. Gan, University of California Riverside; W. Jiang, California Environmental Protection Agency / Department of Environmental Sciences, University of California, Riverside / graduate student, University



of California, Riverside / Environmental Sciences. Hardscape surfaces such as concrete are an important component of urban environments, including residential areas. Insecticides used for structural and landscape pest control, including pyrethroids and fipronil, may be applied directly onto these surfaces, or deposited indirectly via application drift, and water or wind-aided transport. Runoff of pesticide residues from urban hardscapes has been often suspected as the main route by which pesticides enter urban waterways. However, the mechanisms underlying such off-site movement are not well understood. We have carried out several studies and established that loose particles, including soil particles and likely small fragments from cement disintegration, are the main carrier of pesticides in urban surface runoff. Most of the pyrethroids in runoff water from concrete surfaces receiving treatment of various formulations were associated with particles  $> 0.7 \mu\text{m}$ . In addition, dust samples collected randomly from a total of 40 single homes showed the constant presence of a wide range of insecticides and their metabolites on driveways, sidewalks and streets. The levels of pesticides appearing in runoff water and the residues found on sponge swipes were closely correlated, further validating the role of loose particles while simultaneously providing an easy prediction tool. Our findings suggest that to minimize pesticide contamination of urban surface waters, a key is to control offsite movement of loose particles on hardscapes.

**157 Impervious Surfaces as a Potential Source of Biologically Active Pesticide Degradates in Urban Runoff** W. Jiang, California Environmental Protection Agency / Department of Environmental Sciences, University of California, Riverside / graduate student, University of California, Riverside / Environmental Sciences; J. Gan, University of California Riverside. Impervious surfaces such as concrete are identified as an important source of pesticide contamination in urban runoff. However, the occurrence of their degradation intermediates in runoff water has not been studied, especially for the contaminated surfaces after extended environmental exposure. We studied the degradation of two commonly used insecticides, permethrin and fipronil, on concrete and assessed the potential of their biologically active degradates to contaminate surface runoff water. Permethrin degradate 3-phenoxybenzoic acid (3-PBA) and three fipronil degradates (i.e. desulfinyl, sulfide and sulfone) appeared in the runoff water 1 d after pesticide treatment, and they were consistently found in subsequent runoff within 3 months of summer conditions. 3-PBA concentrations were consistently above  $100 \mu\text{g/L}$ , and the runoff half-lives of fipronil degradates were 1.3-2.2 times of parent fipronil. 3-PBA is a common degradate of several pyrethroids and has endocrine disruption activity. The three fipronil degradates have longer environmental persistence than fipronil, and have comparable or greater acute toxicities to arthropods. After 20 d, the concentrations of 3-PBA and total fipronil degradates became higher than their respective parent compounds, indicating the importance to include them in monitoring studies and regulations for urban water protection. Over 70 % of fipronil degradates and 3-PBA were found in the dissolved phase of runoff water, indicating their potential for long-distance transport and bioavailability.

**158 Important Pathways and Effect of Application Practices on Residential Runoff of Pyrethroids** R.L. Jones, Bayer CropScience; P.C. Davidson, C.M. Harbourt, Waterborne Environmental, Inc.; P. Hendley, Syngenta Crop Protection, LLC. Runoff studies to determine the major pathways for transport of pyrethroids applied to suburban residences were conducted at a full scale test facility in central California. Six replicate house lots were typical of front lawns and house fronts of California residential developments and consisted of stucco walls, garage doors, driveways, and residential lawn irrigation sprinkler systems. Each of the six lots also included a rainfall simulator to generate artificial rainfall events. Different pyrethroids were applied to five surfaces—driveway, garage door and adjacent walls, lawn, lawn perimeter (grass near the house walls), and house walls. The volume of runoff water from each house lot was measured, sampled, and analyzed to determine the amount of mass lost from each surface. Applications to three of the house lots were made using the application practices typically used prior to recent label changes, while applications were made to the other three house lots according to the revised application procedures. Results from the house lots using the historic application procedures showed that losses of the compounds applied to the driveway and garage door (including the adjacent walls) were more than 99.5 percent of total runoff losses. The highest losses were associated with significant rainfall events rather than lawn irrigation events. However, runoff losses were 50 times less using the revised application procedures recently specified on pyrethroid labels.

**159 Development and Validation of an Approach for Modeling Pyrethroid Residues in Urban Streams** M.F. Winchell, Stone Environmental, Inc. / Senior GIS Specialist, Stone Environmental, Inc. / Senior Environmental Modeler / GIS Specialist; S.H. Jackson, BASF Corporation; B.N. Toth, J.P. Hanzas, Stone Environmental, Inc.; G.C. Mitchell, FMC Corporation. Pyrethroid insecticides have been detected in urban waterways throughout California and other regions. Modeling pyrethroid environmental fate in a residential setting can help us to better understand the sources, transport pathways, and mitigation strategies for reducing environmental concentrations of pyrethroids. Currently, there is not a standard modeling approach for estimating environmental concentrations of pesticides in an urban environment, either as part of a tiered risk assessment or for development of mitigation strategies. One modeling approach is to adapt an urban water quality model, the Storm Water Management Model (SWMM) to serve this purpose. To test the suitability of SWMM in predicting urban pesticide exposure, the model was applied to a heavily developed residential neighborhood in southern California. This neighborhood was modeled because of the data available from monitoring of pyrethroids conducted there over a several year period. One of the primary inputs to the model, the wash-off characteristics of the pyrethroid, were calibrated to observed plot-scale data for both impervious and pervious turf surfaces. A statewide survey regarding the spatial and temporal patterns of pyrethroid applications was used in structuring the SWMM model to allow realistic application variability throughout the neighborhood. Because of their direct effects on "dry weather" wash-off and storm drain residues, residential outdoor water use practices represented another important component to the model structure and parameterization. The hydrologic component of the model was calibrated to 1 years' worth of hourly flow data measured from a storm drain outfall draining the neighborhood. Pyrethroid samples from both storm and non-storm periods were used to adjust the pesticide components of the model, including application patterns and wash-off characteristics. This presentation will report on the model development, calibration results, and the potential for utilizing this modeling approach as part of a standardized risk assessment process and as a tool for evaluating the potential benefits of best management practices.

**160 Outcomes from Studies on the Transport, Fate and Ecotoxicology of Pyrethroids in Urban Settings** P. Hendley, Syngenta Crop Protection, LLC.; R.L. Jones, Bayer CropScience; M.G. Dobbs, Bayer Crop Science, Bayer CropScience / Ecotoxicology Group; A.C. Barefoot, Du Pont Crop Protection / Crop Protection Products; D. Tessier, E.I. Du Pont De Nemours And Co., Inc.; G. Mitchell, FMC Corporation; S. Jackson, BASF Corporation / Principal Scientist; N. Assaf, Valent BioSciences corporation; J. Hemiller, Amvac Chemical Corporation; K.S. Henry, Syngenta Crop Protection, Inc. / Ecological Sciences. Over the last few years, there has been considerable interest in the occurrence and potential impacts of pyrethroid insecticide residues in sediment and water samples from urban streams. In California, the Department of Pesticide Regulations (DPR) instituted a data re-evaluation on pyrethroids to help refine risk assessments and identify the sources of residues occurring in urban streams and, where necessary, potential mitigation alternatives. Since 2006, the Pyrethroid Working Group (PWG) has been developing data to address DPR's requirements in addition to conducting a wide range of related studies to improve understanding of the science underlying the transport, occurrence and potential impact of pyrethroids arising from residential uses. Laboratory-based PWG studies have included an investigation of sediment fate, acute ecotoxicology studies on *Hyalella azteca* and *Chironomus tentans* as well as continuing improvements in analytical methods. Indoor studies have examined the potential for commercial pyrethroid products to washoff from various building surfaces. Work has also been conducted in model systems and via monitoring to understand how pyrethroids behave in wastewater treatment plants. At the "real world" scale, PWG has conducted multi-year sediment monitoring and deposition area mapping programs in several urban stream systems with complementary bioassessment studies to provide ecological context. Additional water column and sediment sampling is under way in the American River. Finally, a field site has been established for conducting replicated studies to identify those transport pathways which contribute most significantly to residential pyrethroid transport (e.g. driveway treatments). Importantly, this replicated study has demonstrated how recent label changes clarifying precisely how pyrethroids may be applied to hard surfaces around homes will result in extensive reduction in off-site transport during storms. A conceptual model of pyrethroid occurrence and transport

has been developed and refined and will be the focus for explaining how these integrated PWG programs have refined our knowledge and helped to demonstrate the efficacy of some mitigation options. This paper will provide an overview of how these studies have increased understanding of pyrethroid occurrence and behavior in urban settings and have helped identify improved ways of continuing to achieve successful insect control while greatly reducing off-target transport.

**161 Anticoagulant rodenticides are pervasive contaminants of terrestrial birds of prey** J. Elliott, Environment Canada / Pacific Wildlife Research Centre, Science and Technology Branch, Environment Canada / Pacific Wildlife Research Centre, Environment Canada / Wildlife Toxicology, Environment Canada / Canadian Wildlife Service; S. Hindmarch, Environment Canada / Science and Technology; L. Wilson, Environment Canada / Canadian Wildlife Service; F. Maisonneuve, Environment Canada. Previous studies, including our own, have reported a high incidence of liver residues of commonly used second generation anticoagulant rodenticide (SGAR) compounds, in raptors, especially rodent-eating hawk and owl species. A proportion of birds tested commonly exhibit symptoms of anti-coagulant poisoning. In the present study we widened the scope of sampling to include other hawk and falcon species. Of 130 raptors analyzed using high resolution GC/MS/MS from a recent survey of birds collected over the period 2005 to 2011 from southwestern British Columbia, 94% had detectable liver residues of at least one SGAR compound. Barred and Great Horned Owls had the highest incidence of exposure and the greatest residue concentrations; however, 5 of 5 Peregrine Falcons and 5 of 5 Sharp-shinned hawks tested had residues of at least two SGAR compounds, indicating pervasive contamination of the food chains of terrestrial birds of prey. The process by which smaller avian prey of falcons and accipiter hawks are contaminated is not known. Both temporal trends and spatial patterns of SGAR contamination in relation to the degree of urbanization or intensive agricultural activity will be tested quantitatively. We will also extend a previous analysis of the relationships between liver residues of SGARs and diagnosis of anticoagulant poisoning.

**162 Anticoagulant Rodenticide Exposure and Toxicosis in Free-living Birds of Prey Presented to a Wildlife Clinic in Massachusetts, USA, 2006-2010** M. Murray, Tufts Cummings School of Veterinary Medicine / Wildlife Clinic. Mortality among birds of prey from anticoagulant rodenticide (AR) toxicosis has been documented in the United States; however, there is little information on the extent of exposure among specific populations of raptors. Moreover, information on the significance of sublethal AR tissue residues and an understanding of how tissue residues relate to toxicosis are lacking, particularly in wild birds exposed to ARs via their natural prey. The study presented here, conducted at the Tufts Cummings School of Veterinary Medicine Wildlife Clinic in Massachusetts, USA, had multiple aims: to document the proportion of four species of free-living birds of prey (red-tailed hawks [*Buteo jamaicensis*], barred owls [*Strix varia*], eastern screech owls [*Megascops asio*], and great horned owls [*Bubo virginianus*]) admitted to the clinic that died or were euthanized due to the severity of their presenting injuries that had AR residues in liver tissue; to identify and quantify ARs present; to evaluate potential sublethal effects of ARs; to describe ante- and post-mortem signs of toxicosis; and to associate liver AR levels with toxicosis. Of 161 birds tested, 86% were positive for liver AR residues. The second-generation AR brodifacoum was identified in 99% of positive birds. Mortality from AR toxicosis was diagnosed in 6% of birds. No sublethal effects of ARs were detected. No apparent association was found between liver AR level and signs of toxicosis, indicating that individual sensitivity to ARs may vary greatly. The results of this study demonstrate the pervasiveness of ARs, particularly brodifacoum, in the prey of the sampled population during the study period. In addition, the data presented here incorporate ante-mortem signs, post-mortem findings, and AR residue levels to present a more complete picture of the significance of liver residue levels in raptors acquired via a natural route of exposure.

**163 Effects of Anticoagulants on Non-Target Species: Risk Assessments using PBPK Modeling** K. Horak, USDA NWRC; B.A. Rattner, USGS-Patuxent Wildlife Research Ctr / USGS, USGS-Patuxent Wildlife Research Center / Beltsville Laboratory, USGS-Patuxent Wildlife Research Ctr / Beltsville Laboratory; S. Volker, USDA NWRC; J. Johnston, USDA FSIS. Rodents cause significant damage to agricultural crops throughout the world and, when accidentally introduced, these invasive species are a serious threat

in many ecosystems. Anticoagulant rodenticides have long been used to control rodent populations. Although quite efficacious at reducing rodent population size, anticoagulants can pose a risk to non-target wildlife which may be exposed through the consumption of rodents that have ingested anticoagulant bait. In order to generate reliable risk assessments for non-target wildlife, concentrations and half-life values of anticoagulants in target rodents must be accurately estimated. Moreover, a clear relationship between exposure and adverse health outcomes in non-target wildlife must be determined. In a series of studies, several exposure scenarios in wistar rats (*Rattus norvegicus*), bobwhite quail (*Colinus virginianus*), kestrels (*Falco sparverius*) and eastern screech owls (*Megascops asio*) were used to establish time-dependent distributions of the anticoagulant diphacinone in numerous tissues. These data were then used to create physiologically-based pharmacokinetic (PBPK) models for diphacinone in both target mammals and non-target avian species. Through use of these validated models, many of the factors affecting the potential exposure of non-target wildlife to anticoagulant rodenticides can be investigated. Data will be presented illustrating the use of model-generated estimates of tissue anticoagulant concentrations to predict the exposure and risks to non-target species associated with different rodent control strategies.

**164 Monitoring Anticoagulant exposure and toxicity in red foxes with fecal samples** P. Berny, Vetagro-sup, campus vétérinaire / Toxicology; M. Sage, Université de Franche-Comté / Laboratoire Chrono-environnement; I. Fourel, Vetagro-sup, campus vétérinaire / Toxicology; M. Coeurdassier, P. Giraudoux, Université de Franche-Comté / Laboratoire Chrono-environnement. In many countries, the red fox (*Vulpes vulpes*) is particularly affected by secondary poisoning with anticoagulants after field control operations against water voles (*Arvicola terrestris*). There is, however, no specific method available to monitor AVK exposure in the field over large areas and no toxicological data are available concerning sensitivity of foxes to bromadiolone. The aim of this work was to compare excretion kinetics of bromadiolone in fox faeces with clinical and haemostatic effects after repeated exposure to poisoned voles. A sensitive method for the quantification of bromadiolone in fox faeces and plasma was developed, using liquid chromatography combined with mass spectrometry (LC-ESI-MS). The LOD was 0.9 µg/kg and 0.15 µg/L, and the LOQ was 3.0 µg/kg and 0.5 µg/L, in faeces and in plasma, respectively. Captive foxes were fed for 2 or 5 days with water voles spiked with bromadiolone at concentrations close to those measured in the field. Faeces and blood were collected for bromadiolone determination, and blood-clotting tests were performed. After euthanasia (D28), a complete necropsy was performed, and liver samples were analyzed for bromadiolone. Residues were detected in faeces 15 hours after the first exposure. They increased dramatically during the exposure period and then gradually decreased, but remained detectable for at least 26 days after the last exposure. Bromadiolone residues in plasma showed a similar pattern but were no longer detectable 7 to 24 days after the last exposure. Two foxes presented very severe external haemorrhages, requiring the administration of the antidote vitamin-K1. Persistence of AVK residues was also monitored in fecal samples and found to decrease slowly (half-life > 1 month). Bromadiolone residues in faeces and their relationships with exposure and other markers will be discussed. These findings provide a basis for programs aiming to monitor the exposure of wild fox populations to bromadiolone (or potentially other second generation AVK) using non-invasive methods based on standard sampling and analysis of residues in faeces.

**165 Rodenticide Residues in the Environment following a Rodent Eradication Operation on Palmyra Atoll** W. Pitt, USDA APHIS Wildlife Services National Wildlife Research Center / Hawaii Field Station; A. Berentsen, J. Eisemann, S. Volker, USDA APHIS Wildlife Services National Wildlife Research Center; G. Howald, A. Wegmann, Island Conservation. We monitored an application of Brodifacoum 25W Conservation in an attempt to eradicate rats from Palmyra Atoll. Brodifacoum 25W Conservation was aerially broadcast twice over the entire atoll, with target application rates of 80 kg/ha and 75 kg/ha, respectively. These application rates were more than 4 and 8 times, respectively, the application rates typically allowed by EPA under this label. In addition, a supplemental hand broadcast application occurred over a 10 ha area in the on Cooper Island (at a rate of 71.6 kg/ha) in response to a live rat capture. We monitored bait fate, treatment efficacy, and residues in the environment. We systematically checked rodenticide residue levels in hermit crabs, fiddler crabs, geckos, ants, cockroaches, fish, sea water, fresh water, and soil prior to and several

periods up to 30 days after the application of bait. We also opportunistically collected all dead animals found on the atoll and tested them for rodenticide residues. Bait was spread throughout the terrestrial areas of the atoll at the target application rate, but significant quantities of bait also entered the marine environment at distances at least as far as 7m from the shore. We collected 81 potential non-target samples representing 14 species of birds, fish, reptiles and invertebrates for residue analysis. We were able to document the movement of brodifacoum through the environment in primary, secondary and perhaps even tertiary exposure pathways. Levels of brodifacoum were detected in nearly every environmental compartment analyzed. However, given the magnitude of the application we are not surprised by these levels. Our data suggest that even in a short one-month period, brodifacoum residues had begun declining in some biological samples.

**166 Island restoration and the anticoagulant rodenticides: An unholy alliance?** G. Howald, Island Conservation; C. Gill, Coastal Conservation. Islands occupy approximately three percent of the earth's surface but provide key habitat for 15-20% of all plant, reptile and bird species. Approximately 50% of confirmed extinctions have been island species and most of these extinctions were the result of invasive species. One of the most significant are introduced rats, which are responsible for 40-60% of all bird and reptile extinctions. Rodents have been introduced onto 82% of the world's islands which not only negatively affect native plant and wildlife species, but also human health, economies and ecosystem services provided by intact systems. Fortunately for native species, introduced rodents can be removed from islands and ecosystems can recover. This conservation measure is now considered to be one of society's most powerful tools for preventing extinctions and restoring island ecosystems. Over 400 rodent eradication have been completed worldwide, most with the use of the second generation anticoagulant brodifacoum. Brodifacoum has high oral toxicity to rodents, often only requiring a single feeding to achieve mortality. However, it is also highly toxic to other mammals and birds, posing an unwanted hazard to non-target wildlife. Conservation practitioners must seek ways to avoid negatively impacting native biological resources whenever practical. However, with most invasive vertebrate eradications, the potential for eradication success must be balanced by risks to individual non-target wildlife. Although both sides of this balancing equation are important, the need to maximize the chances of eradication success is particularly acute and some non-target mortality (sub-population level impact) may prove unavoidable as long as these individual negative effects can be outweighed by the expected beneficial effects of rat eradication (e.g. recovery of native species and ecosystems). Conversely, a failed eradication attempt resulting from prioritizing a desire to avoid or minimize non-target impacts above all else will provide few conservation returns in the long term because surviving invasive rodents will reproduce at high rates and quickly reoccupy vacant territories throughout the island. Furthermore, a failed eradication attempt will still put individuals of non-target species at risk with no measurable improvements to the species of interest, or the ecosystem as a whole.

**167 Investigating the Risk of Secondary Rodenticide Poisoning to Owls Inhabiting and Foraging in Urban Landscapes of the Lower Mainland, British Columbia** S. Hindmarch, Environment Canada / Science and Technology; J. Elliott, Environment Canada / Pacific Wildlife Research Centre, Science and Technology Branch, Environment Canada / Pacific Wildlife Research Centre, Environment Canada / Wildlife Toxicology, Environment Canada / Canadian Wildlife Service. Anticoagulant rodenticides are widely used to control pest rodents, but poisoning of non-target wildlife has been linked to these practices, including secondary poisoning of birds of prey, particularly owls. In this study, we investigate whether Barred owls (*Strix varia*), Great-horned owls (*Bubo virginianus*) and/or Barn owls (*Tyto alba*) inhabiting and foraging in predominantly urban landscapes of the Lower Mainland, British Columbia are at risk of consuming rodenticide-laden prey, such as rats and house mice. By conducting a pellet study, we found that urban Barred owls had the largest proportion of rats in their diet, with some individuals' diet consisting primarily of rats. Urban Great-horned owl pellets were also comprised mainly of rats, but there was a clear shift towards alternative prey base when urbanization within home ranges decreased. Field voles (*Microtus townsendi*) were the main prey item for Barn owls, regardless of the amount of urbanization within their home range. For all three species, consumption of rats and house mice appears to coincide with increased urbanization within home ranges. The shift in the diet of owls living in urbanized areas may potentially lead to an increased risk of secondary

rodenticide poisoning. Radio telemetry was deployed to further investigate which landscape features urban Barn owls select as foraging habitat and whether they forage in proximity to buildings where rodenticide is applied. Urban Barn owls were found to predominantly forage in grass strips along highway interchanges and verges, and untended grass patches within the city. The majority of foraging was done within 100 m of commercial buildings where rodenticide had been applied. These findings will be discussed in conjunction with previous research done on rodenticide residues found in the livers of deceased owls and the current and historic sales of rodenticides in the Lower Mainland, BC.

**168 Mitigating risk of rodenticides to nontarget wildlife – potential options and unintended consequences** A. Fairbrother, Exponent / EcoSciences, Exponent, Inc. / EcoSciences; A. Kaiser, S. Kane Driscoll, R. Tinsworth, Exponent. Recent studies of liver concentrations indicate that nontarget wildlife, especially owls and hawks, are being exposed to anticoagulant rodenticides. It is desirable to reduce this exposure and potential risk even though the consequences of these exposures are difficult to predict because diagnostic tissue concentrations are not available and necropsy findings of diffuse hemorrhage need careful interpretation. Risk mitigation can occur through the use of less toxic products or by placing fewer baits in locations frequented by nontarget wildlife. Replacing second generation anticoagulants (e.g., brodifacoum or bromadiolone) with first generation products (e.g., warfarin or chlorophacinone) may not reduce risk to all nontarget species because subacute toxicity from repeated exposures would remain high and the development of resistance in rodents to the first generation products may result in increased body burdens. Replacing anticoagulant rodenticides with the neurotoxin bromethalin may increase risk to nontarget species (including children and pets) because bromethalin poisoning is difficult to diagnose (exposure results in nonspecific neurological signs and minimally diagnostic postmortem brain lesions), is difficult to measure in tissue due to extremely rapid photodegradation, has a high bioaccumulation potential, and has no known antidote (in contrast with anticoagulant exposures, which are treatable with Vitamin K). Restricting consumer use of anticoagulants will not significantly decrease wildlife exposure if continual bait placements around commercial establishments, in public parks, or around agricultural buildings (feedlots, chicken houses, etc.) by licensed pest control operators are not also curtailed. Effective strategies for reducing risk to nontarget wildlife may include increasing public awareness of the unintended consequence of rodenticide misuse, providing additional and appropriate training of licensed pest control operators emphasizing implementation of as-needed bait placements, and improving sanitation. Since there is currently no evidence that anticoagulant rodenticides are causing population declines in urban wildlife, it is imperative to take sufficient time to appropriately assess the relative risk of alternative products and approaches in order to select and implement an effective risk mitigation strategy.

**169 Derivation of congener-specific dioxin and furan soil to earthworm bioaccumulation factors** M. Buonanduci, ARCADIS; P. Anderson, Ohio EPA. The uptake of dioxins and furans from soil by earthworms is often a critical assumption and primary risk driver in terrestrial ecological risk assessments as well as the development of cleanup levels and screening benchmarks. We reviewed and summarized all published literature on earthworm uptake of dioxins and furans. Un-normalized and lipid-organic carbon normalized soil to earthworm bioaccumulation factors (BAFs) for 2,3,7,8-tetrachlorodibenzo(p) dioxin (2,3,7,8-TCDD) and the other sixteen 2,3,7,8 substituted congeners were estimated and compared across studies. In cases where the authors themselves did not calculate a BAF, but sufficient information was provided, a BAF was calculated using the data presented in the paper. The review finds that most, but not all, 2,3,7,8 substituted congeners are taken up at a lower rate than 2,3,7,8-TCDD and, therefore, that the BAF for 2,3,7,8-TCDD cannot be applied to the other congeners or the 2,3,7,8-TCDD toxic equivalent concentration in soil. We develop bioaccumulation equivalency factors (BEFs) to account for differences in uptake between congeners. An example of their recommended application is provided. Our review also found that the earthworm bioaccumulation model for 2,3,7,8-TCDD developed by Sample et al. (1998), which is frequently applied in ecological risk assessments and the development of screening benchmarks, is an outlier and overestimates uptake by more than 10-fold.



**170 The influence of soil type on the toxicity of silver to soil microbial processes** K. Langdon, CSIRO / M. McLaughlin, CSIRO / Land & Water, University of Adelaide / School of Agriculture, food and wine, CSIRO / Advanced Materials Transformational Capability Platform, Land & Water; J. Kirby, CSIRO / Advanced Materials Transformational Capability Platform, Land & Water; G. Merrington, WCA Environment Ltd / NCEHS. Silver (Ag) has been shown to exhibit extensive antimicrobial properties, which has led to its increased use in many consumer products, mainly in the form of Ag-nanoparticles. In comparison to many other metals, there is currently little known about the environmental behaviour and potential risks associated with Ag in soils. Although Ag in its ionic form ( $\text{Ag}^+$ ) has been shown to exhibit ecotoxicological properties, it is likely that interactions with the soil matrix will result in variations in Ag bioavailability and toxicity, similar to that seen for other metals. This study examined the toxicity of Ag to the soil microbial nitrification process and assessed the influence that varying soil properties may have on this measure of toxicity. Toxicity testing was conducted in seven soils that showed a wide range of physico-chemical properties, including pH ( $\text{CaCl}_2$ ) (4.6 – 8.0), organic carbon (0.9 – 12%), clay content (1.4 – 60%) and cation exchange capacity (5.3 – 42  $\text{cmol}^+/\text{kg}$ ). Soluble Ag was added to the soils as  $\text{AgNO}_3$  for all toxicity tests, as this provided the most available form of Ag in the soils ( $\text{Ag}^+$ ). Toxicity testing was conducted on soils both leached and unleached, which is the approach used to account for the effects normally observed as a result of spiking soils with soluble metal salts (i.e. increased electrical conductivity and decreased soil pH). The results from this study produced  $\text{EC}_{50}$  values (concentrations corresponding to a 50% effect or inhibition) in the low mg Ag/kg concentration range. However, these toxicity values varied over approximately two orders of magnitude, indicating that there is a strong influence of soil properties on the toxicity of Ag in soils. The results suggest soil organic carbon plays the greatest role in decreasing the toxicity and bioavailability of Ag in soils. Additional toxicity testing with a wider range of organisms is required to confirm these results. The results from this study can be incorporated in the development of Ag-specific terrestrial toxicity relationships, which can be used to predict toxicity in a range of soils, therefore, assisting in the environmental risk assessment of Ag.

**171 Environmental bioavailability of lead in metal-rich granules produced by the earthworm *Eisenia fetida*** R. Lanno, Ohio State University / Department of Evolution, Ecology, and Organismal Biology; S. Brasfield, M. Ansley, US Army Engineer Research and Development Center; J.G. Coleman, US Army Engineer Research and Development Center, US Research and Development Center / Environmental Laboratory, US Army Engineer Research and Development Center / Research Biologist, US Research and Development Center / Research Biologist; J. Seiter, U.S. Army Corps of Engineers / Research Physical Scientist; R. Jones, 2US Army Engineer Research and Development Center; S.R. Bowman, The Ohio State University / Evolution, Ecology, and Organismal Biology, The Ohio State University / Department of Evolution, Ecology, and Organismal Biology, The Ohio State University; M. Vasko-Bennett, Ohio State University. Lead (Pb) is the most common metal contaminant found in the soils of small-arms ranges of US military installations. Currently, risk assessors often assume that Pb is 100% bioavailable for ecological receptors, even though research has shown the modification of Pb bioavailability and toxicity by soil physicochemical characteristics and metabolism by organisms. Many terrestrial invertebrates are capable of rendering Pb toxicologically inactive through the formation of metal rich granules (MRGs). While Pb in MRGs is not considered bioavailable via trophic transfer, little is known regarding the fate of MRGs in the terrestrial environment once released from exposed organisms. The current research characterizes the microstructure of MRGs formed by the earthworm, *Eisenia fetida* and determines the potential for the microbial degradation and remobilization of Pb previously sequestered in MRGs. If liberated by bacterial degradation, sequestered Pb may become bioavailable to invertebrates and their predators. The direct bioavailability of Pb in MRGs to oligochaetes in soil will also be examined. Earthworms were exposed to soils spiked with 4,000 mg/kg Pb for a six-week period and fractionated utilizing differential centrifugation and digestion to separate whole-animal tissue from MRGs to obtain sufficient MRGs for amending soils for microbe and oligochaete exposures. Once extracted, MRG composition and concentration was analyzed utilizing synchrotron-based analysis at Argonne National Laboratories (Argonne, IL). Synchrotron imaging provided multi-channel analyzer (MCA) plots which revealed localized MRG hotspots within the samples, not only confirming the presence of Pb,

but also the binding of additional metals such as zinc and iron. Additionally, synchrotron techniques were utilized on cross-sections of earthworms to determine the presence and concentration of Pb across a gradient of dermal and gut tissue. MCA plots of cross sections revealed a wide dispersion of Pb inside the gut wall, suggesting the presence of unbound Pb in addition to MRG formation. The results of the exposure of microbes and oligochaetes to Pb in MRGs will be discussed.

**172 Derivation of a Tier 2 site-specific remedial objective for Bromacil, a soil sterilant** G.L. Stephenson, Stantec Consulting Ltd. / Environmental Management, Stantec Consulting Ltd. / Environmental Remediation Services, Stantec Consulting / Environmental Toxicology & Remediation; R.A. Angell, E.J. Shrive, Stantec Consulting / Environmental Toxicology & Remediation; K. Bessie, EBA, A Tetra Tech Company / Soil Science; A. Burk, Cenovus Energy. Site-specific soil standards for chemicals are derived to be protective of organisms under specific exposure conditions. Climate change directly and indirectly influences the prevailing environmental conditions such that natural processes are altered (e.g., rates of microbial degradation, adsorption, and bioavailability of chemicals). As a result, chemicals in soil could be more (or less) persistent or bioavailable. Bromacil, a synthetic herbicide, was used in Alberta as a soil sterilant to control vegetation on facilities associated with the oil and gas industry. The rates and frequency of applications, the nature of the chemical, and the increasingly arid conditions in some areas have resulted in concentrations that persist in soil despite the fact that the chemical can be degraded by microbial organisms. These persistent concentrations of Bromacil in soil have the potential to migrate off site and adversely affect crop production or contaminate ground and surface waters. Therefore, the semi-arid lands with persistent Bromacil concentrations in soil require remediation. In Alberta (and many other jurisdictions), there is no soil quality standard for Bromacil to protect ecological receptors exposed as a result of direct contact with contaminated soil. During the decommissioning of a site, concentrations of Bromacil were measured in soils collected across the site and the risk associated with these exposure concentrations was considered unacceptable for ecological receptors. An ecotoxicity assessment with a battery of test methods and species was conducted with both a fine-textured and coarse-textured soil using chemically spiked site soils. Subsequently, species sensitivity distributions of the toxicity data ( $\text{IC}_{25}$ s) were used to derive a site-specific remedial objective (SSRO) for Bromacil in soils for the direct contact exposure pathway. The SSRO was based on the 25<sup>th</sup> percentile of the distribution of inhibiting concentrations ( $\text{IC}_{25}$ s) for plant and soil invertebrate species and plant species alone. Because the sensitivities of the plant species were much greater than those for soil invertebrate species, the recommended SSRO for the eco-contact exposure pathway was based on the SSD for plant species only.

**173 Arctic mites and nitrifiers: should these drive our remediation clean up standards** S.D. Siciliano, A. Schafer, University of Saskatchewan / Toxicology Group; J. Princz, Environment Canada / Ecotoxicology and Wildlife Health Division; J. Owjori, University of Saskatchewan. The derivation of site-specific remedial objectives is a key component of any remediation plan. For many sites, human receptors are not routinely present and thus, ecological receptors or off site migration, drives clean-up standards. We have developed a new invertebrate toxicity test species, oribatid mites, as well as validated a biogeochemical toxicity test: potential nitrification which can be used to set ecologically based clean up standards. Both of these new tests have been compared to standard toxicity tests and found to compare well. In addition, we have modified the mite and biogeochemical toxicity tests to allow rapid, on-site assessments of toxicity. The goal of these modifications is to allow front-line personnel to rapidly (< 24 hr) assess the toxicity of a soil sample and thereby provide guidance for clean up efforts. However, both of these tests suffer from limitations in their use. Specifically, oribatid mites are sensitive to organic matter content, and in some cases, nitrification inhibition may not be a relevant endpoint for the ecosystem. I will discuss the current state of polar region soil toxicity testing, how it applies to remediation designs, and what I think are some reasonable next steps to allow us to develop ecoregion specific remedial objectives.

**174 A new processing pipeline and policy framework to incorporate pyrotag sequencing results into soil toxicity testing and assessments in Polar Regions** S.D. Siciliano, University of Saskatchewan / Toxicology Group; J. van Dorst, B. Ferrari, University of New South Wales; K. Mengersen, Queensland University of Technology; J. McKinley, B.

Raymond, C. King, I. Snape, Australian Antarctic Division. Risk assessment of soil sites in Polar Regions are dominated by microbial activities because on many of these sites there are no higher animals or vascular plants. Traditional soil activity toxicity testing, such as respiration or nitrification, are sensitive indicators of change. Further, the application of molecular approaches such as Quantitative PCR is clearly within our current paradigm of how sites should be assessed for toxic impacts. However new technologies, specifically massively-parallel sequencing efforts, such as the Ion Torrent or 454 platforms, provide new opportunities into how a site can be assessed and characterized. Here, we present our view of how the results from these sequencing technologies can be processed and interpreted. Specifically, we will outline the processing pipeline developed in R to handle these datasets as well as the policy framework necessary to incorporate these results into a risk assessment. Finally, using an 18 month chronic exposure study to diesel pollution, we will provide an example of how this new pipeline and framework compares to traditional approaches to soil toxicity assessment.

**175 Earthworm responses to petroleum hydrocarbons in aged field contaminated soil using both traditional ecotoxicity endpoints and  $^1\text{H}$  NMR metabolomics** M. Whitfield Aslund, Intrinsik Environmental Sciences Inc., University of Toronto; G.L. Stephenson, Stantec Consulting Ltd. / Environmental Management, Stantec Consulting Ltd. / Environmental Remediation Services, Stantec Consulting / Environmental Toxicology & Remediation; M.J. Simpson, University of Toronto / Department of Physical and Environmental Sciences.

$^1\text{H}$  NMR metabolomics was used to examine the metabolic profile of earthworms exposed to petroleum hydrocarbons (PHCs) in soil. Significant relationships were observed between the earthworm metabolic profile (collected after two or 14 days of exposure) and soil properties including soil PHC concentration, suggesting that earthworms responded to PHC contamination at a metabolic level. Since the ecological relevance of metabolomic responses is currently poorly understood, soils were also evaluated using more traditional earthworm ecotoxicological endpoints. Although not acutely toxic to earthworms (average survival  $\geq 90\%$ ), some soils impaired reproduction endpoints by  $>50\%$  compared to the field control soil. Further comparisons by partial least squares regression revealed a significant relationship between these reproduction endpoints (collected after 63 days) and earthworm metabolomic data (collected after only two or 14 days), which suggests it may be possible to develop metabolomics as a rapid tool to predict chronic toxicity of PHCs in soil to earthworms.

**176 Risk Assessment of petroleum hydrocarbon contamination in soil.**

**II. Toxicity of conventional and renewable fuels to three test species** V. Malk,

University of Helsinki / Palmenia Centre for Continuing Education; E. Barreto Tejera, Autonomous University of Madrid / Department of Biology; O. Penttinen, University of Helsinki / Department of Environmental Sciences. Oil and fuel spills are among the most extensive and environmentally damaging pollution in Finland and other Nordic countries, but only limited data on the ecotoxicology of petroleum hydrocarbons exist. Petroleum products, such as diesel fuel and gasoline, are complex mixtures of compounds when their toxicity depends on composition of fuel and joint effect of harmful constituents. Composition of new environmentally friendly products such as biodiesel and ethanol based gasoline can be expected to have different toxicity profile when compared to conventional gasoline and diesel. Oil products exist in soil pores as non-aqueous phase liquids (NAPL) which is challenge for ecotoxicity testing. Organisms may be affected by oil as a result of physical fouling or by exposing to toxic compounds. For determining effects of toxic compounds alone, water soluble fractions (WSF) of fuels need to be used in ecotoxicity testing. In this study, ecotoxicological effects of conventional gasoline and diesel, as well as RE85 gasoline and renewable NExBTL diesel in soil were determined by exposing earthworms (*Eisenia fetida*) during 14 days in a laboratory. Acute toxicity of water soluble fractions of different fuels was determined by exposing water fleas (*Daphnia magna*) and luminescent bacteria (*Vibrio fischeri*). WSFs were generated by mixing one part of fuel to four parts of water by 22-24 hours. NExBTL had lower percentage proportion of heavy hydrocarbon fractions (C21-C40) and PAH compounds in test soil when compared to conventional diesel. EC50 value for diesel was 1600 mg/kg while for NExBTL toxicity was not detected at concentration of 5000 mg/kg. The co-solvency effect of ethanol increased the solubility of BTEX compounds and short chained PHCs (C5-C10) in the RE85 WSF solution. EC50 for *Vibrio fischeri* in RE85 and gasoline exposure was approximately 1800 mg/l and 8300 mg/l.

*Vibrio fischeri* was considerably less sensitive for PHC compounds than *Daphnia magna*.

**177 Modeling Mercury Distribution in the Sarasota Bay Ecosystem using SERAFM and Stable Isotope Ratio of Nitrogen ( $\delta^{15}\text{N}$ )** Y. Hong,

Johns Hopkins University / Geography and Environmental Engineering, Johns Hopkins University; E. Rifkin, National Aquarium Baltimore; E. Bouwer, Johns Hopkins University / Geography and Environmental Engineering. Mercury (Hg) transport and transformation and Hg bioaccumulation in the Sarasota Bay ecosystem are investigated using the Spreadsheet-based Ecological Risk Assessment for the Fate of Mercury (SERAFM). The mercury biomagnification from the foodchain base to the blood of Sarasota Bay resident bottlenose dolphins is modeled using a linear relationship between logarithmic Hg concentrations and stable isotope ratio of nitrogen ( $\delta^{15}\text{N}$ ) in biota. By combining and using the two models, the Hg sources of the bay are identified and the strategies to lower the reported methylmercury (MMHg) levels in the blood of resident bottlenose dolphins are investigated. The model is calibrated with field data collected by the authors in the Sarasota Bay during the summer of 2011. The combined model successfully related atmospheric total mercury (THg) deposition to the Hg concentrations in the overlying water, sediment, various shellfish and finfish species, and in the blood of resident bottlenose dolphins. The model showed that the THg loading to the bay is about  $7.6 \text{ kg yr}^{-1}$ . The Hg runoff from impervious areas in the watershed and the THg atmospheric direct deposition to the waterbody were the two major Hg sources to the bay occupying 46% and 31% of the THg loadings, respectively. To lower the average MMHg concentrations in the blood of resident bottlenose dolphins from  $570$  to  $150 \text{ ng g}^{-1}$  (the reference value observed in the bottlenose dolphins of Charleston, SC), the THg loadings may need to be reduced by 74% from  $7.6$  to  $2.0 \text{ kg yr}^{-1}$ . The reduction can be achieved by lowering the THg atmospheric deposition from  $20.6$  (value of 2010) to  $3.0 \mu\text{g m}^{-2} \text{ yr}^{-1}$ . If appropriate watershed implementation plans could reduce the Hg runoff from the impervious surface by 50%, then the current THg loading to the bay can be decreased by 26% from  $7.8$  to  $5.8 \text{ kg yr}^{-1}$ . Under such condition, the reduction of atmospheric THg deposition to  $4.5 \mu\text{g m}^{-2} \text{ yr}^{-1}$  is required to lower the Hg blood levels in the Sarasota resident bottlenose dolphins to the value observed in Charleston, SC. The increasing population in the Sarasota Bay watershed is likely to increase the Hg runoff from impervious areas, so appropriate storm water management together with the reduction of Hg atmospheric deposition may be required to reduce the Hg levels in the bay ecosystem.

**178 Seasonal and annual trends in forage fish mercury concentrations,**

**San Francisco Bay** B.K Greenfield, University of California, Berkeley, University of California, Berkeley / Berkeley Center for Green Chemistry; A.R. Melwani, R.M. Allen, San Francisco Estuary Institute; D.G. Slotton, S.M. Ayers, University of California – Davis / Department of Environmental Science and Policy; K.H. Harrold, University of North Carolina / Environmental Sciences and Engineering; K. Ridolfi, LimnoTech; M.B. Sandheinrich, University of Wisconsin – La Crosse / River Studies Center, University of Wisconsin – La Crosse / Department of Biology. Monitoring of mercury (Hg) in San Francisco Bay forage fish has revealed relatively elevated concentrations throughout the Bay ecosystem. As a result, potential short and long-term changes in Hg exposure and bioaccumulation have been of interest among Hg researchers. In 2005, the Regional Monitoring Program for Water Quality in San Francisco Bay began monitoring Bay forage fish to indicate temporal patterns in bioaccumulation. Interannual variation and long-term trends were determined by monitoring Hg bioaccumulation during September – November, for topmelt and Mississippi silverside at six sites, over a six year sampling period (2005 – 2010). Seasonal variation was characterized for arrow goby (*Clevelandia ios*) at one site and topmelt at four sites during 2008 – 2010. Statistical model evaluation indicated both seasonal and interannual variation to be present. Seasonal patterns accounted for 30 – 60% of the total variation in arrow goby and topmelt Hg concentrations. Arrow goby exhibited a consistent seasonal pattern from 2008 – 2010, with lowest concentrations observed in March or April, and highest concentrations in July or September. In contrast, topmelt concentrations tended to peak in late winter or early spring. The seasonal patterns were not correlated with changes in body size, but may relate to seasonal shifts in abiotic concentrations in the contrasting habitats of the two species. In general, strong directional trends for Hg in forage fish were not apparent over the study duration. For both species, the variability explained



by year was relatively low compared to sampling station, suggesting that interannual variation is not a strong influence on Hg concentrations. Topsmelt (*Atherinops affinis*) exhibited a significant increase in Alviso Slough from 2005 to 2010 that was not observed at other locations, which may relate to ongoing habitat restoration in adjacent salt ponds. Although fish Hg has shown long-term declines in some ecosystems around the world, San Francisco Bay forage fish did not decline over the six year monitoring period examined.

**179 Hg bioaccumulation in shoots of the macrophyte *Elodea nuttallii* happens through copper transporters** N. Regier, University of Geneva; C. Cosio, Geneva University, Geneva University / Forel Institute. Previous studies suggest that macrophytes might participate in bioaccumulation and biomagnification of toxic mercury (Hg) in aquatic environment. Hg bioaccumulation and uptake mechanisms in macrophytes need therefore to be studied. Amongst several macrophytes collected in an Hg contaminated reservoir in Romania, *Elodea nuttallii* showed a high organic and inorganic Hg accumulation and was then further studied in the laboratory. *Elodea nuttallii* is a rooted submerged aquatic macrophyte native of North America, which occurs as aggressive invader worldwide in temperate areas. It is often found in metal contaminated sites in freshwater environments. Hg tolerance and accumulation of this plant was also high in the microcosm. Basipetal transport of inorganic Hg was predominant, whereas acropetal transport of methyl-Hg was observed with apparently negligible methylation or demethylation *in planta*. In addition methyl-Hg proportion as total Hg was increased in rhizospheric sediments. Hg concentrations were higher in roots>leaves>stems and in top>middle>bottom of shoots. In shoots, more than 60% Hg was found intracellularly where it is believed to be highly available to predators. Accumulation of inorganic and methyl-Hg in shoots was highly reduced by cold, death and by competition with Cu<sup>+</sup> for inorganic Hg. Hg in *E. nuttallii* shoots seems to mainly originate from the water column, but methyl-Hg was produced in rhizosphere and can also be remobilized from the sediments driving in part its entry in the food web. At the cellular level, uptake of Hg into the cell sap of shoots was linked to the metabolism, in particular to copper transporters. In summary, our results revealed that shoots of *E. nuttallii* are extremely tolerant to Hg and show huge bioaccumulation ability. Based on densities previously reported in the field by other authors (350-2800 g DW m<sup>-3</sup>) and concentration of Hg found in Babeni reservoir, it can be estimated that 0.7 to 5.6 mg Hg m<sup>-3</sup> could be accumulated in these plants. This amount is likely to return to water and sediments at the end of the growing season when plants decay. Moreover, the Hg uptake by shoots might be an important pathway of Hg from the water to organisms feeding on the macrophytes. In conclusion, these plants should probably be removed in contaminated sites.

**180 Bottom-up nutrient and top-down fish impacts on mercury flux from aquatic ecosystems** M.M Chumchal, T. Jones, R. Drenner, Texas Christian University / Biology department; W.H. Nowlin, Texas State University / Department of Biology; G. Timmins, Texas State University / Biology department. Methyl mercury (MeHg) is one of the most hazardous toxins in the environment, adversely affecting the health of wildlife and humans. Recent studies have demonstrated that aquatic insects biotransport MeHg and other contaminants to terrestrial consumers, but the factors that regulate the flux of MeHg out of aquatic ecosystems via emergent insects have not been studied. We used experimental mesocosms to test the hypothesis that insect emergence and the associated flux of MeHg from aquatic to terrestrial ecosystems is affected by both bottom-up nutrient effects and top-down fish consumer effects. In the present study, nutrient addition led to an increase in Hg flux primarily by enhancing the biomass of emerging insects whose tissues were contaminated with MeHg, while fish decreased Hg flux primarily by reducing the biomass of emerging insects. Further, we found that these factors are interdependent such that the effects of nutrients are more pronounced when fish are absent and the effects of fish are more pronounced when nutrients concentrations are high. The present study is the first to demonstrate that the flux of Hg from aquatic to terrestrial ecosystems is strongly enhanced by bottom-up nutrient effects and diminished by top-down consumer effects.

**181 Spatial and temporal variations in fish mercury concentrations in Grand Lake, Oklahoma, USA** L.A. Schaidt, Harvard School of Public Health / Department of Environmental Health; Z. Dong, Harvard School of Public Health / Environmental Health; R.A. Lynch, University

of Oklahoma Health Sciences Center / Occupational and Environmental Health; S. Ziara, Grand River Dam Authority; J.P. Shine, Harvard School of Public Health / Department of Environmental Health. Exposure to methylmercury (MeHg) through fish consumption is a public health concern, with thousands of fish consumption advisories in the U.S. Coal-fired power plants are the primary anthropogenic sources of atmospheric mercury (Hg) emissions and freshwater bodies near these plants may contain elevated Hg in sediments and biota. This study is based at Grand Lake, northeastern Oklahoma, a reservoir located within 100 km of six coal-fired power plants. As part of a community-based participatory research project to evaluate MeHg exposure among residents who consume local fish, we measured Hg concentrations in commonly-consumed fish throughout the watershed, evaluated food web dynamics using  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  and tested for spatial and temporal variations in Hg concentrations in prey fish and plankton. This project addresses community concerns about Hg exposure through consumption of local fish and involved local agencies and recreational fishermen in the collection of fish. Total Hg was measured in 700 filet samples representing 23 types of commonly-consumed fish. In general, Hg concentrations were below the U.S. EPA tissue residue criterion of 0.3  $\mu\text{g g}^{-1}$  MeHg, with the exception of several flathead catfish, drum and largemouth bass. While Hg concentrations were generally higher as  $\delta^{15}\text{N}$  increased, elevated Hg in some flathead catfish could not be explained by  $\delta^{15}\text{N}$ , suggesting they derive their dietary Hg from a different food web. Hg concentrations were correlated with fish length for some types of fish, but showed no length dependence in other types. While differences in Hg concentrations were not observed in higher trophic level fish among different parts of the watershed, fish at lower trophic levels may more closely reflect spatial and temporal variations in MeHg production. Samples of prey fish (minnow, brook silver-side), young mid- and high-level consumers (largemouth bass, sunfish) and plankton (53  $\mu\text{m}$ , 163  $\mu\text{m}$ , 363  $\mu\text{m}$ ) were collected monthly throughout spring and summer from two locations in Grand Lake (an upstream location with primarily riverine conditions and a downstream location with primarily lacustrine conditions) and from one location in Lake Hudson, another reservoir immediately downstream of Grand Lake. Samples were analyzed for total Hg,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , and a subset was also tested for MeHg. These results provide insight into the extent that Hg concentrations in fish reflect localized variations in MeHg production.

**182 Environmental factors controlling mercury methylation in low productivity sediments** P. Schierz, university of Texas at Austin / Department of Civil and Environmental Engineering, Duke University / Pratt School of Engineering, Duke University / Civil and Environmental Engineering, university of Texas at Austin / Department of Civil, Architectural and Environmental Engineer, The University of Texas at Austin / Center for Research in Water Resources; P. Bireta, J.S. Grundy, L.E. Katz, The University of Texas at Austin / Civil, Architectural and Environmental Engineering Department-EWRE; D.D. Reible, The University of Texas-Austin, University of Texas / Environmental and Water Resources, The University of Texas at Austin / Dept. of Civil & Environmental Eng.. Mercury is a pollutant of global concern. In sediment environments, inorganic mercury can be transformed to methylmercury, the most dangerous form, by sulfate and/or iron reducing bacteria. Strongly reducing, organic rich sediments typically exhibit maximum methylation at the transition between iron reduction and sulfate reduction. The purpose of this study is to evaluate mercury methylation behavior in low productivity, deep water sediments and to identify key controlling factors. Here we define low productivity sediments as sediments low in labile organic matter that exhibit low sediment oxygen demand and are only mildly reduced with limited or minimal sulfate reduction. Sediment batch slurry experiments were performed with marine, mercury contaminated sediment under different geochemical conditions initiated by different amendments such as Fe<sup>2+</sup>, sulfate and lactate. The slurry experiments with no amendments as well as iron and sulfate amendment were characterized by mildly reduced conditions reflected in low sulfate reduction (as indicated by acid volatile sulfides, AVS) after 78 days. Slow accumulation of AVS indicated continued slow reduction and reflected the low productivity of the sediment. The addition of an electron donor (lactate) stimulated microbial activity in the sediment slurries, increased reduction (fully reduced conditions) and methylation. However, this does not reflect field site conditions. Results identify labile organic carbon as the limiting factor for mercury methylation under field site conditions. Mesocosms studies (overflow cells) were also performed to explore representative field conditions (density, vertical redox gradient) using voltammetry, DGT (diffusive gradient in thin



films) pore water analysis to characterize geochemical parameters, mercury availability and net methyl mercury production. Results of these laboratory studies were compared to the analysis of field cores (both frozen and refrigerated) to verify reproduction of field conditions. The limited reduction in the sediments resulted in low methylmercury concentrations (< 0.1% of mercury in porewater). The observed MeHg levels are consistent with observations in the raw sediment from the site, indicating that the experimental systems are representative of site conditions and suggesting that the site poses much less risk (in terms of methylation potential) than would normally be ascribed to the observed bulk solid mercury concentrations.

### **183 Selenium:mercury interactions in aquatic systems receiving anthropogenic inputs of mercury and selenium**

**A.R. Stewart**, U.S. Geological Survey / Water Resources Division; **D. Krabbenhoft**, J. DeWild, C. Thompson, U.S. Geological Survey. Global fish consumption advisories to protect humans against mercury (Hg) toxicity are at odds with the positive nutritional benefits of consuming fish for their high quality protein and omega-3 fatty acids. This conflict has led to discussions within regulatory and scientific communities about strategies for reducing Hg exposures in nature and mitigating its toxic effects, including the role of selenium (Se). Some studies suggest that when Se is present in molar quantities greater than Hg Se may reduce the bioaccumulation of Hg and protect against Hg toxicity at a physiological level. Yet understanding of specific mechanisms controlling molar Se:Hg ratios in nature are far from clear. Interactions between the elements may be occurring at geochemical, physiological and ecological levels. Here, we present results on Se-Hg interactions at two different ecological settings (San Francisco Bay, CA; and Goose Lake, MI), but share a common condition of being distinctly contaminated by mining and/or agricultural practices. In San Francisco Bay we evaluated the bioaccumulation of Hg and Se in top predator fish. Molar Se:Hg ratios in fish muscle tissue ranged from 3-29 in adult white sturgeon muscle, 3-15 in juvenile striped bass, and 1-6 in adult striped bass. Variation in Se:Hg molar ratios among individuals and developmental stage appeared to be related to their foraging location relative to Se sources in San Francisco Bay. These results indicate that while all predators analyzed in the study had molar Se:Hg > 1 some ratios did approach 1 and all varied widely within and among species in an estuarine environment with spatially variable sources of Se and Hg. At Goose Lake, field studies were designed to evaluate whether antagonistic Se-Hg interactions would limit Hg availability for methylation, and subsequent incorporation into the aquatic food web. Our results show that there was very little apparent inhibition effect of Se on methylation of ambient Hg or tracer-level amendments of  $^{202}\text{Hg}$  used for quantifying methylation. However, while methylmercury was clearly produced, unlike most aquatic ecosystems, very little was passed up the food chain beyond the primary producer level, resulting in very low Hg levels at higher trophic levels. These studies indicate that interactions between Se and Hg are complex and that further research on processes controlling bioaccumulation across a range of Hg and Se exposure levels and environmental settings is warranted.

### **184 Bioenergetic effects of food web simplification on mercury biomagnification in northern pike (*Esox lucius*) in southern Alberta, Canada**

**L. Brinkmann**, University of Lethbridge; **J. Rasmussen**, University of Lethbridge / Department of Biological Sciences. In the past, studies on mercury contamination in reservoirs have focused on the pulse in baseline levels following inundation and ensuing responses in resident biota. Considerably less is known about the trophodynamic and bioenergetic effects on mercury biomagnification in communities responding to the lotic-lentic transition. Absence of key taxa or functional groups in aquatic communities can lead to food web simplification and inefficient energy transfer between lower trophic levels and top predators. Low growth efficiency, or bioenergetic bottleneck effects, may be reflected in stunted growth of species exploited by humans, recreationally or commercially. Growth efficiency of fish is also a key determinant in the biomagnification of contaminants such as mercury, in that it affects the accumulation of body tissue for a given amount of contaminant assimilated during feeding. This study investigates mercury biomagnification in relation to growth characteristics of northern pike in lakes and reservoirs of semi-arid southern Alberta, Canada. These systems differ in their forage fish community, in that zooplanktivorous lake whitefish, an important link between pelagic and littoral food webs, are either present (LKWH+) or absent (LKWH-). In LKWH- systems diets of northern pike consist predominantly of invertebrates (amphipods and leeches), despite the presence of other potential forage fish species. In LKWH+ systems lake whitefish is a

major constituent in the diet of northern pike. Stable isotope analysis ( $\delta^{15}\text{N}$ ) shows that food chains are shorter in LKWH- systems, placing northern pike at lower trophic position compared with LKWH+ systems. Based on previous work we expect flattened growth trajectories of northern pike in LKWH- systems, reflecting lower growth efficiency, relative to LKWH+ systems. We hypothesize that achieving saturation on an invertebrate diet imparts significant activity costs on large pike and therefore reduces growth efficiency of pike in these systems. In conjunction with reduced growth efficiency we expect to find greater mercury biomagnification factors of pike from their invertebrate diet. In the greater context bioenergetic constraints resulting from food web simplification may compound the risk of mercury contamination in fish following construction of new reservoirs. We propose that community structure, trophodynamics and bioenergetics be addressed in the planning of reservoir projects.

### **185 Using macrobenthic community structure to identify stressors in Southern California estuaries, bays, and harbors**

**D. Gillett**, J.A. Ranasinghe, M. Satula, Southern California Coastal Water Research Project; **D. Cadien**, County Sanitation Districts of Los Angeles County; **K. Schiff**, Southern California Coastal Water Research Project. Macrobenthic community structure is a commonly used measure to assess general environmental condition in marine and freshwater habitats. When degraded conditions are detected, it then becomes necessary to identify the cause(s) leading to the impact. Compared to assessments however, there has been comparatively minimal progress towards the development of macrobenthic-based tools for the diagnosis of individual stressors that lead to degraded condition. Eutrophication and chemical contaminants are the most pervasive stressors impacting the quality of estuaries and coastal oceans around the world. Though they are often the product of different anthropogenic activities, little work has been done to develop approaches to monitoring/assessment that differentiate between the two. Our goal was to develop practical tools that use macrobenthic community structure to help diagnose the major stressor(s) impacting the sediments and overlying waters of the estuaries and coastal ocean of the Southern California Bight. We developed suites of potential indicator taxa from 4 years of regional monitoring data and tested their ability to predict sediment eutrophication (elevated TOC and TN) or chemical contamination (e.g., metals, organics, pesticides). In preliminary analyses, several taxa show promise. They are being tested for independence from natural gradients and will be validated against a new, naive dataset. Eventually, macrobenthic community metrics developed from these indicator taxa could be included in the state of California's coastal and estuarine monitoring tools. These additions should provide valuable stressor insights that could be used in causal assessments of locations with degraded benthic communities.

### **186 Stressor identification analysis in a small salmon-spawning watershed in the Sacramento Valley**

**B.S. Washburn**, OEHA/Cal EPA / Ecotoxicology Program; **K.A. Pulsipher**, Ecotoxicology Program / Office of Environmental Health Hazard Assessment; **W.G. Wieland**, Ecotoxicology Program / Office of Environmental Health Hazard Assessment. The Stressor Identification (SI) methodology, based on principles of eco-epidemiology, was used to pinpoint the cause(s) of the decline in fall-run chinook salmon in a HUC 10 watershed northeast of Sacramento. The SI process involves listing candidate stressors, analyzing data from the case and from other situations, and characterizing causes based on a set of criteria. The dataset used for the analysis was collected from ten different sites in the watershed over a period of 6 years. Due to the complexity of salmon's life cycle, benthic macroinvertebrates (BMI) were used as a surrogate assessment endpoint. Relationships between water quality, physical habitat, land use characteristics, and BMI metrics were analyzed. We developed a scoring system to evaluate data from the watershed as well as data from elsewhere. Over fifty possible stressors were evaluated. Most were eliminated because they failed to meet basic criteria; a dozen were advanced for detailed analysis. The percent silt, sand, and fine gravel was most closely linked to the decline in the abundance and diversity of BMIs, with a total score of 44 out of a possible 55. A variety of physical habitat and conventional water quality parameters were weakly correlated with BMI metrics. The percent impervious cover in the 100 ft. buffer in the stream network was the landscape factor most closely linked to poor BMI metrics. Conversely, the percent open space within a 100 ft. buffer in the stream network was strongly linked with healthier BMI populations. The results of the analysis will be used to prepare a watershed indicator report.

**187 Satellite Image Analysis to Assist Remedial Action Decisions**

W. Stiteler, Arcadis U.S., Inc.; C. Meyer, A. Thatcher, ARCADIS. Soil remediation is necessary in areas of high contamination, but in many cases remediating an area may do more harm than good to existing vegetation, particularly in arid areas. This study presents the results of an analysis of land cover type and health using satellite remote sensing. The study area consists of over 40,000 acres, making intensive sampling of vegetation health through ground sampling impractical. Ikonos satellite imagery of the area, consisting of information in the visible and near-infrared portions of the electromagnetic spectrum, was used to classify the quality of rangeland and wildlife habitat using three metrics. This information was used to target areas for remediation from within an estimate of areas of high contamination based on geostatistical analysis. The first metric, rangeland condition for cattle, was assessed using a maximum likelihood classification of the imagery, trained with a set of ground samples. The second metric, vegetative cover for wildlife was estimated with a model using a vegetative index calculated for each Ikonos pixel, calibrated with field visits. The third metric, wildlife habitat quality, was found to correlate with species richness. This was assessed with the mean and variance of the vegetation index across a twenty-five square meter area, which provided a measurement of both vegetative cover and heterogeneity. Another maximum likelihood classifier was used on this aggregate index data and trained using field measurements at a similar spatial scale to determine which areas had acceptable habitat quality. Accuracy from ground sampling was 70% for rangeland condition, 80% for vegetative cover, and 74% for species richness assessments. Areas with cover and species richness meeting 60% of that of reference areas, and rangeland sites in fair or good condition are likely to not need remediation.

**188 Using Receiver Operating Characteristic Analysis To Characterize Stressor-Response Relationships for Causal Analysis in Ecological Risk Assessment**

D.B. McLaughlin, National Council for Air & Stream Improvement / Northern Regional Center, National Council for Air & Stream Improvement / Western Michigan University. The analysis of field-derived stressor-response data plays a key role in building evidence to support conclusions regarding the presence of a causal link between a potential stressor variable and a biological or ecological response of interest. Several data analysis methods are widely available for describing a stressor-response relationship and its associated uncertainty. For example, USEPA's Causal Analysis/Diagnosis Decision Information System (CADDIS) website includes information on regression analysis, classification and regression tree analysis, conditional probability analysis, propensity score analysis, and others. Receiver operating characteristics (ROC) analysis is one relatively simple data analysis approach that, while not new to ecological research, appears to be under-utilized in causal assessment. More commonly used in medical diagnosis, machine learning, and other fields, ROC analysis quantifies the strength of relationships between true and indicated or predicted conditions (e.g. the presence or absence of disease based on the results of a diagnostic test). Applied to ecological risk assessment, ROC analysis can be used to identify a level or range of stressor necessary to maintain a desired ecological condition, and to characterize the uncertainty in predictions of ecosystem state based on levels of a stressor. One advantage of ROC analysis over other methods is its comprehensive quantitation of false positive, false negative, positive predictive, and negative predictive errors when comparing the predictive performance of alternative candidate models of ecosystem or biological condition. In addition, characterizing model performance in these terms can be more meaningful to decision-makers and affected stakeholders than mean square error, regression  $r^2$ , or other terms from more common statistical procedures. In turn, the overall strength of evidence supporting the presence of a causal relationship can be more transparent to a broad audience. Software for conducting ROC analysis is available using the open source R statistical package. In this presentation, important elements of ROC analysis will be presented along with examples using field-derived stressor-response data in which ROC results are compared with results from conditional probability analysis and linear regression.

**189 A Bayesian network approach for causal inferences in pesticide risk assessment and management** J.F. Carriger, Jr., U.S. EPA, U.S. EPA / Gulf Ecology Division; M.G. Barron, US EPA / Gulf Ecology Division. Pesticide risk assessment and management must balance societal benefits and ecosystem protection, based on quantified risks and the strength of the causal linkages between uses of the pesticide and socioeconomic and ecological endpoints of concern. A Bayesian network (BN) is a graphical representation

of the joint probability distribution over a set of discrete random variables and provide a mathematical approach for assessing causality. A BN can represent the best available knowledge of causal dependencies between variables important to risk management such as the influence of application events on contaminant exposure. Capabilities, advantages, and disadvantages of BNs to enhance assessments of causality in pesticide risk assessment and management are demonstrated through a model development process. The process focuses on the incorporation of domain knowledge to represent and evaluate cause-consequence relationships between system variables and endpoints, and the integration of non-causal factors important to a risk problem such as measurement uncertainty, definitional interactions, and reconciliation of independent models. For the causal linkages, conditional and marginal probabilities in the BN represent the strength of evidence on causal associations and permit the incorporation of a wide range of statistical model output and expertise. The model building process illuminates the potential for BNs to facilitate the identification of new management options, improve risk communication, assimilate competing hypotheses, and enhance public participation in risk management tasks. Some of these features of BNs are exhibited in a case study of lampricide risks and benefits in Great Lakes region. The example BN will demonstrate predictive reasoning on the efficacy and potential risks to non-target organisms from several uses and diagnostic reasoning for updating beliefs on potential causes from observed effects.

**190 A Causal Analysis of Mercury Impacts to Invertebrates in a High-Gradient Freshwater Stream**

J.R. Flanders, URS Corporation, URS; G. Long, URS; W.H. Clements, Colorado State University / Fish, Wildlife and Conservation Biology, Colorado State University, Colorado State University / Fish, Wildlife and Conservation Biology; R.G. Stahl, DuPont Company / Corporate Remediation Department, DuPont Company / Corporate Remediation Group, DuPont Company / DuPont Corporate Remediation Group. Mercury (Hg) is a persistent bioaccumulative contaminant that may present potential ecological risk to wildlife on a regional basis, but the effect of direct contact exposure to aquatic invertebrates is difficult to gauge due to high stochasticity in invertebrate communities, variation in species sensitivity, and multiple potential stressors. A causal analysis of invertebrate community exposure to Hg in the South River, VA, which has high Hg concentrations in surface water, sediment and biota relative to other regional watersheds was conducted between 2006 and 2011. The causal analysis used empirical field data and *in situ* experiments (e.g., benthic colonization and *in situ* microcosm approaches), laboratory toxicity tests (sediment quality triad), statistical modeling, and data from other systems to determine potential impacts to invertebrate communities. Field experimental data indicated that the relative composition of functional feeding groups and major classes or orders of invertebrates were not substantially different between study sites and pooled reference areas, and that there were no observed population- or community-level differences between study sites and reference areas. A comprehensive multivariate statistical analysis of invertebrate community, physical habitat and water chemistry and quality data revealed that invertebrate abundance and species richness were determined by a complex interaction between physicochemical variables, substrate composition and Hg; only one taxon had Hg concentration as a primary explanatory variable. Sediment quality triad data revealed that there was no toxicity or effects on the invertebrate community at concentrations up to 18.9 µg/g total Hg (THg) or 102 ng/g methylmercury (MeHg). An evaluation of data from other freshwater sites from the SEDTOX database revealed no association with toxicity at sites with concentrations ranging up to 949 mg/g THg. The findings of these lines of evidence were further supported by sediment pore water analyses, which indicated that concentrations of THg and MeHg in sediment pore water samples from the South River were not consistent with concentrations associated with adverse effects to invertebrates in aqueous toxicity tests reported in the literature. The conclusion of the causal analysis is that although invertebrates themselves may contribute to the mercury uptake by other organisms, there is little if any direct risk to invertebrates in the South River.

**191 Causal Assessment of Biological Impairment in a Low Gradient Coastal California River: The Salinas River** S. Hagerthey, S. Norton, U.S. Environmental Protection Agency / National Center for Environmental Assessment. Biological impairment of California perennial streams is defined by a region specific benthic macroinvertebrate index of biological integrity (B-IBI) score. For the central coast, the southern California coastal IBI (SoCal B-IBI) is used, with values less than or equal to 39 being categorized

as "poor" and deemed as not meeting the biological objective. In 2006, biological assessment of four sites along the middle and lower Salinas River, Monterey County, had scores below the SoCal B-IBI impairment value of 39. The lower river sites had scores of 14 and 19 and were categorized as "very poor". In contrast, the low gradient middle river sites had scores greater than 24 and were considered "poor". We conducted a causal assessment that utilizes a pragmatic approach based on the U.S. Environmental Protection Agency's Stressor Identification Protocol ([www.epa.gov/caddis](http://www.epa.gov/caddis)) to identify the likely cause of the "very poor" IBI scores. Stakeholders assisted in identifying seven potential candidate causes. These were changes or alterations in dissolved oxygen, pesticides, metals, nutrients, ionic strength, flow, sediments, and physical habitat. A conceptual diagram and narrative were constructed for each candidate cause to link with potential sources and effects. Evidence from the case (i.e., data specific to the river) and from elsewhere (i.e., not specific to the river) were weighed against comparator sites to develop a "strength of evidence" score for each candidate cause. Scores were then used to identify the probable cause(s). Preliminary scoring suggested that dissolved oxygen and ionic strength were not the likely impairment causes. Pesticides, metals, nutrients, flow, sediments, and physical habitat could not be ruled out and were evaluated further using a weight of evidence approach. The advantages of conducting a causal assessment when biological objectives are not met are that it is scientifically defensible formal method, reproducible, prevents biases, may identify unexpected causal relationships, engages stakeholders and decision makers, and increases confidence that management actions will improve biological condition. **DISCLAIMER:** The views expressed in this presentation are those of the authors and do not necessarily reflect the views or policies of the U.S. Environmental Protection Agency.



**192 Leaching of Urban Biocides from Building and Construction**

**Materials: Use of LC-MS/MS for Detection in Storm Water Runoff and Wastewater** N. Stock, Trent University / Water Quality Centre; C.D. Metcalfe, Trent University / Environmental & Resource Studies, Trent University / Department of Environmental & Resource Studies. Urban biocides, particularly those used in building and construction materials that have the potential to leach to the aquatic environment during rain storm events, have recently become a class of contaminants of emerging concern. A liquid chromatography – tandem mass spectrometry (LC-MS/MS) method has been developed to analyze for three target urban biocides: Terbutryn, Mecoprop and Irgarol 1051. A series of samples including storm water runoff, wastewater, and receiving water from Southern Ontario were analyzed, and ng/L concentrations of the three target urban biocides were observed. To confirm identity of these a quadrupole – time of flight (QTOF) mass spectrometry method for accurate mass measurements and investigation of isotopes was also developed.

**193 Screening Wastewater Effluent and River Water for Emerging Micropollutants using a Multi-residue GC-MS-AIQS Database method**

M. Allinson, University of Melbourne / CAPIM; F. Shiraiishi, D. Nakajima, National Institute for Environmental Studies; G. Allinson, Department of Primary Industries, Victoria / Future Farming Systems Research Division. Urban municipal wastewaters may contain a large number of organic chemicals, some of which, when discharged to receiving waters, may prove directly toxic to organisms living therein, while others may elicit more subtle effects, including eliciting endocrine disrupting effects. Managing the effects of such contaminants ultimately requires information on effluent toxicity and chemical concentrations. Preliminary screening of samples using rapid assessment tools is an increasingly attractive prospect for treatment plant and waterways managers. Rapid screening does not preclude the innovative use of more traditional techniques, such as gas chromatography – mass spectrometry (GC-MS). Kadokami et al. (2005) developed a new method combining a mass-structure database with GC-MS (the Automated Identification and Quantification System with GC-MS Database system, or GC-MS-AIQS-DB) that can screen samples for more than 900 semi-volatile compounds, including numerous halogenated and non-halogenated hydrocarbons, polynuclear aromatic hydrocarbons (PAHs), polychlorinated biphenyl compounds (PCBs), and range of pharmaceutical and personal care products (PPCPs), and pesticides. In 2007, samples of treated effluent were collected at point of discharge to the environment from 40 WWTPs located across Victoria, Australia. The wastewater treatment plant samples investigated in this study were prepared using relatively straightforward process involving solid-phase extraction, and then tested using the GC-MS-AIQS-DB method. Chemicals observed in one or more effluents included food additives (dibenzylether, 98% of the 40 effluents), various alkyl phenols (4-*t* octylphenol, 20%; bisphenol A, 13%), tyre leachates (e.g. 2(3H)-benzothiazolone, 40%), flame retardants (TBP, 68%; TCEP, 83%), antioxidants (BHT-aldehyde, 45%), insect repellents (DEET, 93%), stimulants (caffeine, 18%) and anticonvulsants (carbamazepine, 68%). Of the 451 pesticides screened, carbamate insecticides (bendiocarb, 33%, propoxur, 40%), and herbicides (simazine, 35%) were amongst the compounds observed. By using the GC-MS-AIQS-DB screen, a single analytical method can be used to cover the large number of compounds, reducing the concomitant financial implications associated with operating multiple definitive tests.

**194 Quaternary ammonium compounds as improved tracers of sewage affected particles and sediments as well as potential contaminants of emerging concern**

A.C. Doherty, Stony Brook University / School of Marine and Atmospheric Sciences, Stony Brook University / graduate student; X. Li, Connecticut Agricultural Experiment Station / Analytical Chemistry; B.J. Brownawell, Stony Brook University / School of Marine and Atmospheric Sciences, Stony Brook University / Marine Sciences Research Center. Studies of quaternary ammonium compounds (QACs) in sediments from urbanized estuaries near the New York metropolitan region indicate that they are typically present in higher abundances than classes of synthetic organic compounds. Total concentrations of 20 targeted QACs average approximately 30 µg/g in muddy sediments of Jamaica Bay, western Hempstead Bay and other areas of the Lower Hudson Basin. Owing to high sensitivity of analysis afforded by electrospray ionization mass spectrometry, a large number of QAC analytes can be very easily detected at distances up to 70 km from sewage sources. Although little studied in the field to date, elevated concentrations alone do not necessarily indicate that QACs should

be classified as CECs. These cationic surfactants merit further investigation due to the antimicrobial properties possessed by some QACs (e.g., benzalkonium compounds and shorter alkyl chain dialkyldimethylammonium compounds (DADMACs)), where concentrations in sediments are appreciably higher than the more extensively studied triclocarban, triclosan and fluoroquinolone antibiotics. It may be that strong sorption of QACs to sediments limits influence on microbial communities. Other reasons for further studies of QACs include the fact that the more abundant and hydrophobic DADMACs (fabric softeners) and the newly discovered behentrimonium compounds (hair care products) may be uniquely suited as source specific tracers of sewage affected particles and their sorbed contaminants. While the hydrophobicities of the 20 targeted QACs vary by many orders of magnitude, the persistence of QAC tracers is inferred by similarities in composition between suspended particles collected at a sewage outfall and in sediments at variable distances away, as well as studies of their persistence in sediment cores. In recent years significant attention has been paid to the application of more soluble organic wastewater contaminants as sewage source indicators, yet many chemical and microbial contaminants of concern are primarily transported associated with sediments or particulate phases. In this presentation we compare and contrast what we know about the properties and behavior of QACs with other proposed tracers of sewage derived particles, as well as illustrate applications where QACs appear to provide useful source allocation information for particle reactive contaminants such as di-ethylhexyl phthalate and brominated flame retardants.

**195 What Goes Down the Drain Eventually Reaches the River: Characterizing Emerging Contaminants in the Columbia River Basin**

J. Morace, USGS Oregon Water Science Center, US Geological Society; E.B. Nilsen, USGS Oregon Water Science Center / OWSC. Toxic contamination is a significant concern in the Columbia River Basin in Washington and Oregon. To help water managers and policy makers in decision making about future sampling efforts and toxic-reduction activities, a reconnaissance was done to assess contaminant concentrations directly contributed to the Columbia River through wastewater-treatment-plant (WWTP) effluent and stormwater runoff from adjacent urban environments and to evaluate instantaneous loadings to the Columbia River Basin from these inputs. Nine cities were selected in Oregon and Washington to provide diversity in physical setting, climate characteristics, and population density. Samples were collected from a WWTP in each city and analyzed for anthropogenic organic compounds, pharmaceuticals, PCBs, PBDEs, organochlorine or legacy compounds, currently used pesticides, mercury, and estrogenicity. Of the 210 compounds analyzed in the WWTP-effluent samples, 112 (53 percent) were detected, and the detection rate for most compound classes was greater than 80 percent. Despite the differences in location, population, treatment type, and plant size, detection frequencies were similar for many of the compounds detected among the WWTPs. By contrast, the occurrence of PAHs was sporadic, and PCBs were detected at only three WWTPs. With a better understanding of the presence of these contaminants in the environment, future work can focus on developing research to characterize the effects of these contaminants on aquatic life and prioritize toxic reduction efforts for the Columbia River Basin. One example is an interdisciplinary project designed to assess contaminants and characterize habitats in the lower Columbia River basin provides an example of this type of research. Using a foodweb approach, emerging contaminants, particularly PBDEs and endocrine-disrupting compounds, are being determined in osprey (a fish-eating raptor), the fish they eat – largescale suckers (*Catostomus macrocheilus*, a bottom-feeding fish), the water column, streambed sediment, and benthic invertebrates. Multiple fish biomarkers, including those focused on size, reproduction, and organ function, provide an assessment of the potential biological effects of these contaminants. The ultimate goal is to provide information about contaminant distributions in the system and contribute to understanding how emerging contaminants are affecting the ecosystem and the foodweb in the lower Columbia River.

**196 Pharmaceuticals and Personal Care Products (PPCPs) in a Wastewater Receiving Stream During Normal and Low Flow Conditions**

A.J. Jones, Clemson University / Institute of Environmental Toxicology, Clemson University / Institute of Environmental Toxicology; E. Carraway, Clemson University / Department of Environmental Engineering and Earth Sciences. The Reedy River, located in the piedmont of South Carolina, is a stream receiving effluent from two wastewater treatment facilities (WWTP) serving the greater metropolitan area of Greenville, SC. The work presented

here details two sample collections during a normal flow condition and a low flow condition. EDCs and NSAIDs can result in negative effects on aquatic populations at very low concentrations (ng/L, pg/L) in surface waters. Variability in pharmaceuticals and PPCPs under low flow situations has been poorly studied. Sample enrichment was accomplished with solid phase extraction (Waters Oasis HLB) and the samples were analyzed using liquid chromatography – tandem mass spectrometry (Varian 320 MS). Analytes of interest are caffeine, diphenhydramine, ibuprofen, naproxen, triclosan, estrone, 17 $\beta$ -estradiol, and 17 $\alpha$ -ethynylestradiol. The results indicate significantly higher concentrations of all analytes, with the exception of estrone, during low flow conditions. In addition, caffeine and diphenhydramine were found in the Reedy at locations upstream of the two WWTP outfalls. Generally decreasing analyte concentrations on a downstream gradient from the WWTP inputs to the stream suggest some removal mechanisms which may include sorption and degradation.

**197 A novel approach to describe the total PPCP contamination in aqueous environments** J.L. Conkle, W. Wang, J.O. Sickman, University of California Riverside / Environmental Sciences; J. Gan, University of California Riverside. There are numerous studies documenting the presence of pharmaceutical and personal care products (PPCPs) in the environment, specifically near wastewater effluent, septic discharge and large-scale agricultural operations. However, there is no general method to quantify total PPCP contamination levels of a water sample, compare effectiveness of treatment technologies or to simplify statistical analysis of complex, variable data sets within or across studies. In this presentation we propose multiple empirical equations to describe the total PPCP contamination in aqueous samples. Each equation was developed using concentrations from 21 previously published studies (with 284 total samples) on PPCPs in aquatic environment. We then applied the best-suited equation to a survey of PPCPs in surface and groundwater of Beaumont, CA. PPCP contamination was observed in surface water samples and multiple groundwater wells as a result of septic discharge, managed aquifer recharge and riverbank filtration. Index values of surface water samples downstream of a wastewater treatment plant discharge were similar to those observed in the review of literature for wastewater effluent, which is the main source of water in this tributary. Additionally, groundwater nearby this tributary reflected the level of PPCP contamination observed in the overlying surface water. Index values observed in groundwater elsewhere in the basin were lower, reflecting contamination from the state water project and septic systems. Through the examination of additional literature, we also present multiple proofs of concept to demonstrate the utility of index values to compare water treatment technologies within and across studies, the ability to compare sample collection, extraction and analytical techniques and also the ability to simplify statistical analysis of large PPCP datasets.

**198 Occurrence of pharmaceuticals and their metabolites in urban aquifers of Barcelona metropolitan area. Impact of recharge sources.** D. Barcelo, ICRA / Environmental Chemistry, ICRA / Catalan Institute for Water Research, IDAEA-CSIC / Environmental Chemistry Department, Catalan Institute for Water Research (ICRA) / Environmental Chemistry Department; R. Lopez-Serna, IDAEA-CSIC; M. Petrovic, ICRA – Catalan Institute for Water Research. Groundwater is an important resource of water supply in the world and urban aquifers are especially vulnerable to contamination by a variety of contaminants. Groundwater in urban aquifers from the Barcelona metropolitan area suffers pollution from different sources, including losses from water supply networks, leakage from sewers, direct rainfall recharge, recharged from the river Besos and seawater intrusion. Previous study showed that contaminants, such as surfactants and their degradation products (i.e. nonylphenol ethoxylates (NPEO), nonylphenol carboxylates (NPEC), nonylphenol (NP) and linear alkyl benzene sulfonates (LAS)) are present at high concentrations (mg/L level) in aquifers recharged by the river Besos showing clear dependence on the sources of recharge or/and pollution containing these substances and ground water redox conditions. Similarly groundwater in the Barcelona area was found to contain low concentrations of drugs of abuse. The highest groundwater concentrations (around 200 ng/L) and the largest number of detected compounds were found in zones recharged by a river that receives large amounts of effluents from wastewater treatment plants. This study reports on the occurrence of pharmaceuticals and their metabolites. 95 compounds belonging to different classes are analyzed in 37 urban groundwater samples collected in the city of Barcelona using optimized and validated on-line solid phase

extraction-liquid chromatography-tandem mass spectrometry (SPE-LC-MS/MS) method. A wide range of pharmaceuticals are detected being the predominant contaminants the most consumed anti-inflammatory drugs (naproxen, ibuprofen, diclofenac),  $\beta$ -blockers (metoprolol), lipid regulators (gemfibrozil) and antibiotics (azithromycin, erythromycin, ofloxacin, sulfadiazine). It is important to mention finding of several metabolites (in some cases in concentrations higher than of parent compounds), such as carbamazepine metabolites 10,11-epoxy carbamazepine, 2OH- and 3OH carbamazepine, desmethyl diazepam, N-acetyl sulfadiazine, 4OH diclofenac, 4OH propranolol and enalaprilat which shows the importance of monitoring metabolites and transformation products in order to properly assess the risk posed by pharmaceutical compounds. Acknowledgements: Authors would like to thank the Spanish Ministry of Economy and Competitiveness for its financial support through the project SCARCE (Consolider-Ingenio 2010 CSD2009-00065)

**199 Halogenated byproducts generated through wastewater treatment in secondary effluent** D.N. Bulloch, University of California Riverside / Chemistry, University of California Riverside; A. Ramkishun, University of California Riverside / Chemistry; C.K. Larive, University of California Riverside / Department of Chemistry; D. Schlenk, University of California-Riverside / Department of Environmental Sciences; J. Armstrong, Orange County Sanitation District / Environmental Compliance & Monitoring. The study of disinfection by-products of pharmaceuticals in wastewater is an emerging field. Most by-products of known contaminants are unregulated, and may have altered physiochemical properties and toxicity profiles compared with the parent. Our previous work determined the levels of halogenated byproducts of the lipid regulator gemfibrozil as generated by primary wastewater treatment followed by chlorination, with a monochlorinated analog occurring at 166 ng/L and a monobrominated analog occurring at 50 ng/L in a final effluent grab sample. Herein this research is extended to measuring the levels of several pharmaceutical and personal care products and their halogenated analogs as potentially formed by chlorination treatment in a secondary effluent grab sample from a Southern California wastewater treatment plant. The parent compounds are the lipid regulator gemfibrozil, the analgesic salicylic acid, the surfactant 4-nonylphenol, the non-steroidal anti-inflammatory diclofenac, and the plasticizer bisphenol A, all of which have been reported in the literature to form halogenated analogs upon exposure to chlorine. In addition, the octanol-water partition coefficients of each parent compound and their halogenated analogs were determined by a microcentrifuge tube-based shake-flask protocol. Experimental partition coefficients for the halogenated analogs are compared to calculated values as generated by the US EPA's Estimation Programs Interface Suite.

**200 TINE: The fate, behavior, and ecotoxicology of manufactured nanomaterials in terrestrial ecosystems** P.M. Bertsch, University of Kentucky / Department of Plant and Soil Sciences; S. McGrath, Rothamsted Research / Soil Science Department; J.M. Unrine, University of Kentucky, University of Kentucky / Department of Plant and Soil Sciences, University of Kentucky / Department of Plant & Soil Sciences, University of Kentucky / Savannah River Ecology Laboratory, University of Kentucky / Department of Plant and Soil Sciences, University of Georgia / Savannah River Ecology Laboratory; O.V. Tsyusko, University of Kentucky / Department of Plant & Soil Sciences; N.J. Kabengi, D.M. McNear, University of Kentucky / Plant and Soil Sciences; G.V. Lowry, Carnegie Mellon University / Dept. of Civil and Env. Engineering, Center for the Environmental Implications of NanoTechnology; E. Casman, Carnegie Mellon University; M. Wiesner, Duke University / Civil and Environmental Engineering; J. Liu, Duke University; A. Neal, Rothamsted Research; B. Jefferson, R. Dorey, K. Ritz, J. Harris, S. Rocks, Cranfield University; S. Lofts, NERC Centre for Ecology & Hydrology / Shore Section; D. Spurgeon, NERC Centre for Ecology and Hydrology; C. Svendsen, Centre for Ecology & Hydrology, Monks Wood / Pollution and Ecotoxicology, NERC Centre for Ecology & Hydrology / Hails Section, NERC Centre for Ecology & Hydrology / Ecotoxicology; H. Zhang, Lancaster University. We have developed a life cycle perspective inspired conceptual model (CM) that indicates terrestrial ecosystems as a major repository for ZnO, TiO<sub>2</sub>, and Ag manufactured nanomaterials (MNMs) introduced via the land application of MNM-containing biosolids. In this project we are investigating the transport, fate, behavior, bioavailability, and effects of MNMs in (to) agroecosystems under environmentally realistic scenarios organized around three key hypotheses: Hypothesis (H1)

Surface chemistry is the primary factor influencing the fate and transport of MNMs in the terrestrial environment as well as the bioavailability and effects to biological receptors; Hypothesis (H2) Once released to the environment, pristine MNM surfaces will be modified by interactions with organic and inorganic ligands (macromolecules) or via other biogeochemical transformations (aging effects forming a-MNMs); Hypothesis (H3) Ecoreceptors will respond to interactions with pristine metal and metal oxide MNMs, a-MNMs, and/or dissolved constituent metal ions and bulk oxides by specific ecological and toxicogenomic responses that will reflect their combined effects. The overall objectives are to: O1) Compare the transport, fate, behavior, bioavailability, and effects of MNMs, a-MNMs, and/or dissolved free metals/bulk oxides to organisms with key terrestrial ecosystem functions, as well as exposure pathways involving humans; O2) Determine MNM, surface modified MNM and a-MNM interactions with important biological targets relevant to the BLM and pBRM models and relate these interactions to physicochemical properties; O3) Validate models with information generated from experiments designed to address O1 for MNMs introduced through a pilot scale Waste Water Treatment Process (WWTP) to key terrestrial ecoreceptors, including effects of MNMs on the WWTP itself; O4) Determine realistic MNM emission scenarios for Tier 1 MNMs in wastewater from the WWT pilot plant data and develop first generation Life-Cycle-Analysis-inspired Risk Assessment (LCA-RA) model components for terrestrial effects of Tier 1 MNMs and a-MNMs based on data generated in experiments designed to address O1, O2, & O3; and O5) Provide tools for in situ detection, monitoring, and characterization of pristine MNMs and a-MNMs in environmental media and biota. The key results from aging and toxicity studies will be presented.

#### **201 Chemical Weathering of Ag and ZnO Nanoparticles in the Presence of Inorganic Sulfide or Inorganic Sulfide/Phosphate Aqueous Environments**

G.V Lowry, Carnegie Mellon University / Dept. of Civil and Env. Engineering, Center for the Environmental Implications of NanoTechnology; R. Ma, Carnegie Mellon University / Civil and Environmental Engineering, Center for the Environmental Implications of NanoTechnology (CEINT); S. Rathnayake, University of Kentucky; J. Stegemeier, Carnegie Mellon University; C. Levard, Stanford University; J. Unrine, P. Bertsch, University of Kentucky. Benthic environments will be a sink for many manufactured nanomaterials (MNMs), especially for MNMs (e.g. Ag(0) and ZnO) used in health and hygiene products because they enter wastewater streams, partition to sewage sludge, and then are used as a soil amendment in agro-ecosystems. MNMs made from class B metals are likely to be readily transformed in sulfidic environments. In biosolids, competing ligands (e.g. phosphate) may further alter the speciation, and in turn alter the particle properties, e.g. solubility and toxicity potential. To assess risk we must understand these weathering reactions and identify the stable weathering products as these are what organisms will be exposed. This work, conducted as part of the Transatlantic Initiative for Nanotechnology and the Environment (TINE), determines the morphology, speciation, and properties of Ag and ZnO NPs in the presence of sulfide, and determines the speciation of ZnO NPs in the presence of the competing ligands sulfide and phosphate. Both Ag and ZnO NPs readily sulfidize upon exposure to inorganic sulfide. Partially sulfidized Ag and ZnO NPs form by using a range of metal to sulfide ratios. Partial sulfidation significantly decreases the solubility of Ag NPs but not ZnO NPs. This suggests that Ag NPs are sulfidized directly without significant dissolution, but that ZnO NPs dissolve followed by precipitation of ZnS. This is confirmed by formation of a coherent Ag-sulfide shell on Ag NPs, but a ring of 4nm ZnS particles surrounding ZnO NPs. Upon exposure to phosphate, ZnO is readily phosphorylated, but with sulfide also present they are primarily sulfidized. The factors that affect phosphorylation vs. sulfidation of ZnO in biosolids are being investigated. Overall, these studies suggest that both ZnO and Ag NPs will be readily sulfidized in biosolids where ample sulfide is present and the system is anoxic, but that the speciation of Zn in biosolids may also include Zn-phosphate (e.g. hopeite) if the redox conditions change. These studies also indicate that a range of Ag(0)/Ag<sub>2</sub>S and ZnO/ZnS ratios can be synthesized in the laboratory. These particles may be more representative of those materials found in the environment useful for subsequent toxicity testing.

#### **202 Comparison of the fate and impact of dissolved and nanoparticle metals on sewage treatment and biosolids production**

B. Jefferson, B. Martin, M. Pawlett, R. Dorey, S. Rocks, K. Ritz, J. Harris, Cranfield

University. Manufactured nanomaterials are increasingly used in consumer products with a large proportion discharged to wastewater treatment facilities leading to the potential transmission pathways into both aquatic and terrestrial ecosystems. Recent estimates suggest that up to 90% of some nanomaterials may end up in sewage highlighting the importance of a clear understanding of their fate so that effective risk assessments can be developed. Whilst there is a growing intensity of studies on the topic information related to real sewages and the ageing effects of NP residence within sewage matrices remains limited. The proposed presentation will outline current activities as part of a joint UK-US consortium investigating the fate and impact of nanoparticles in sewage biosolids. Three identical pilot sewage treatment facilities have been set up to treat real raw sewage incorporating sedimentation, activated sludge, thickening, anaerobic digestion and dewatering processes to mimic the processes most likely to be encountered. A mixture of titanium, silver and zinc nanoparticles are being dosed into both the primary sludge and the activated sludge components prior to mixing and biosolids production in anaerobic digestion and dewatering. One of the other identical lines is dosed with an equivalent concentration of dissolved metals to ascertain any NP specific effects and the final line is operated without dosing as a control. All plants are operated under standard conditions and were stabilised for three sludge ages prior to start of analysis. Comparison of the partitioning rates into the final bio-solid product revealed that in the case of titanium and zinc less total metal associated with the biosolids in the NP dosed line compared to the metal salt dosed line. In contrast, in the case of silver more of the total metal content associated to the sludge in the NP dosed line. No statistical difference between NP dosed and metal salt dosed lines could be observed in terms of organics removal, nitrification, solids destruction, biogas generation although difference in the microbial community within the NP dosed lines were apparent. Current work is fractionating the different liquid streams across the treatment lines to establish the colloidal banding of the metals and this data will also be presented.

#### **203 Effects of soil chemistry of zinc and zinc oxide NP toxicity to the earthworm *E. fetida***

S. Lofts, NERC Centre for Ecology & Hydrology / Shore Section; L. Heggelund, E. Lahive, NERC Centre for Ecology and Hydrology / Hails Section; D. Spurgeon, NERC Centre for Ecology and Hydrology; C. Svendsen, Centre for Ecology & Hydrology, Monks Wood / Pollution and Ecotoxicology, NERC Centre for Ecology & Hydrology / Hails Section, NERC Centre for Ecology & Hydrology / Ecotoxicology. Metal and metal oxide nanoparticles present a series of challenges for terrestrial ecotoxicology. Chemical transformations of the nanoparticle may modify the exposure of organisms to nanoparticles. Additionally, the chemistry of the soil itself may influence exposure, for example by promoting particle aggregation or dissolution. Within the TINE project, we aim to develop models to take account of these processes, based on sublethal exposures of zinc oxide (ZnO) and silver (Ag) particles to the earthworm *Eisenia fetida* across a range of soil types. The exposure model will account for (i) particle dissolution and consequent toxicity of the ionic metal across soil types; (ii) variation in the toxicity of undissolved nanoparticles across soil types. Initial results will be shown from earthworm exposures to ZnO nanoparticles, non-nano ZnO and ionic Zn, in a set of soils created in the laboratory by liming of an acidic heathland soil from the southern UK. Initial results show that (i) exposure to all three types of Zn result in effects on earthworm reproduction; (ii) the toxicity consistently increases with decreasing soil pH; (iii) ZnO, both nano and non-nano, is consistently less toxic on a Zn basis than ionic Zn; (iv) the toxicity in ZnO exposures cannot be solely attributed to ionic Zn formed by particle dissolution; (v) accumulation of Zn by the worms is higher in particle exposures than in ionic exposures. Taken together, these findings suggest that direct nanoparticle toxicity to earthworms does occur in soil exposures. The differences in uptake and toxicity between ZnO particles and ionic Zn suggest that (i) particles are directly taken up by earthworms; (ii) uptake does not trigger zinc elimination processes in the worm, hence the relatively high accumulation of Zn in particle exposures; (iii) on a Zn basis, particles are less toxic to worms than ionic Zn once within the tissues. We will discuss these findings in the context of developing the concepts behind the earthworm-nanoparticle exposure model.

#### **204 nanoBEE; a joint US-UK project to model exposure, bioavailability and toxicity of nanoparticles**

J. Lead, University of Birmingham / School of Geography, Earth and Environmental Science. This talk will give an overview of the nanoBEE project which aims overall to validate a set of



exposure, bioavailability and toxicity models so that they are both scientifically valid and usable by regulators and industry. After the scene setting, the synthesis of size and surface coating selected silver nanoparticles will be discussed. Finally, the presentation will focus on the behaviour of these nanoparticles in toxicology media over suitable time periods which mimic standard exposure times. Dynamic changes in particle properties such as aggregation, oxidation, dissolution and surface changes will be examined as will the effects of media modification. Changes in dose and the nature of the toxicant in media will be highlighted and related to toxicity studies under the same media conditions.

#### 205 Environmental Exposure and Toxicity of Engineered Nanomaterials

V. Colvin, Rice University; P.J. Alvarez, Rice University / Civil & Environmental Engineering; E. Contreras, H. Puppala, Rice University. In order to understand and develop models that can predict the environmental fate of nanomaterials (NMs) in the environment well characterized NMs are required. With this aim in mind our group has developed an organic synthetic approach for oleic acid stabilized silver nanocrystals without employing an additional size separation technique. The diameter of the nanocrystals can be controlled over a wide range from two to fifteen nm with narrow size distribution ( $\sigma < 15\%$ ). Facile ligand exchange of oleate with thiol functionalized methyl-poly(ethylene glycol) (mPEG-SH, MW 5KDa) transformed silver nanocrystals to hydrophilic with retention of optical properties. Further, stability of pegylated silver nanocrystals in high ionic media extends its applications to environmental studies involving complex matrices. The work presented herein will discuss our synthetic technique and the use of these NPs to understand factors that influence the stability and dissolution of Ag NPs (e.g. size, medium, etc). In addition to understanding the NMs physical and chemical properties, to fully understand the biological and environmental impacts of nanomaterials requires studies which address both sub-lethal endpoints and multigenerational effects. Here we use a nematode to examine these issues as they relate to exposure to two different types of QDs, core (CdSe) and core-shell (CdSe/ZnS), and compare the effect to those observed after cadmium salt exposures. The strong fluorescence of the core-shell quantum dots allows for the direct visualization of the materials in the digestive track within a few hours of exposure. Multiple endpoints, including both developmental and locomotive, were examined at QD exposures of low (10 mg/L Cd), medium (50 mg/L Cd), and high concentrations (100 mg/L Cd). While the core-shell QDs showed no effect on fitness (lifespan, fertility, growth, and three parameters of motility behavior), the core QDs caused acute effects similar to those found for cadmium salts. Over multiple generations, we commonly found that for lower life-cycle exposures to core quantum dots the parents response was generally a poor predictor of the effects on progeny. At the highest concentrations, however, biological effects found for the first generation were commonly similar in magnitude to those found in future generations.

#### 206 Tracing bioavailability of metal oxide nanoparticles using stable isotope labeling

T.S. Galloway, University of Exeter / Biosciences Department; S. Dogra, University of Exeter; F. Larner, Imperial College; A. Dybowska, Natural History Museum; E. Valsami Jones, University of Birmingham; M. Rehkamper, Imperial College; J. Lead, B. Stohlpe, University of Birmingham; C. Tyler, University of Exeter. Metal oxide nanomaterials have a wide range of uses in industrial applications and consumer products, and there is a pressing need for risk assessments for human and environmental health. For many metals, high background concentrations in natural systems hinder the interpretation of exposure studies at environmentally relevant exposure concentrations. Aim: To study the bioaccumulation and biological effects of ZnO nanoparticles (ZnO NPs) in a model invertebrate test species, using stable isotope tracing methods with high detection sensitivities. Methods: The amphipod *Corophium volutator* (mud shrimp) is an OECD test species and constituent of the marine food web.  $^{68}\text{ZnO}$  NPs were synthesised using wet chemistry. We exposed *C. volutator* in simple microcosms to DEG- $^{68}\text{ZnO}$  NPs (diameter 30nm), and  $^{68}\text{ZnO}$  in bulk and soluble forms for 10 days, and evaluated the partitioning of particles/ionic Zn between water column, sediment and body tissues. Coherent anti-stokes Raman spectroscopy (CARS), TEM and EDX helped establish the uptake and tissue distribution of particles *in situ*. Multiple collector ICP-MS was used to evaluate the Zn isotopic ratios in samples. Results: Our results show that uptake of  $^{68}\text{Zn}$  from particles into storage tissues of the organism is active, and appears to proceed through normal physiological processes. Comparable work with  $^{109}\text{Ag}$  is underway and we will also present our latest results with

this material. Conclusions: Our work illustrates the sensitivity of stable isotope tracking for studying nanoparticles in complex environmental settings and offers insight into the biological methods used to detoxify metal oxides and NPs *in vivo*.

#### 207 The utility of human ADME data for prioritizing the evaluation of pharmaceuticals in the environment

J. Berninger, Baylor University / Department of Environmental Science; C. LaLone, U.S. EPA; C. Russom, U.S. EPA / Mid-Continent Ecology Division; J.E. Tietge, US EPA / Office of Research and Development, National Health and Environmental Effects Research Laboratory, Mid-Continent Ecology Division; D.L. Villeneuve, U.S. EPA / National Health and Environmental Effects Research Laboratory; G.T. Ankley, US EPA / Office of Research and Development, National Health and Environmental Effects Research Laboratory, Mid-Continent Ecology Division. To proceed in the investigation of potential effects of pharmaceuticals in the environment, a cohesive data collection strategy is paramount. Given the lack of data for aquatic species, prioritization seems a logical starting point. Several methods have been put forward, for example using predicted persistence, bioaccumulation, and toxicity characteristics or sales data models. While these models are valid, critical prioritization strategies, they are not sufficiently comprehensive. Pharmaceuticals are biologically active, with specific physiological targets, suggesting that models built solely on physical-chemical properties and exposure potential cannot be adequate for prioritization. Given the nature of pharmaceuticals, the overall prioritization should include chronic effects and ADME (absorption, distribution, metabolism, excretion) parameters. The lack of available aquatic species data requires the development of an ADME-based prioritization model using read-across from the extensive mammalian knowledge-base. ADME data for 750 drugs were collected including: apparent volume of distribution, clearance rate, half life, protein binding, and therapeutic dose. For this initial prioritization, a probabilistic model was applied to data from each parameter, and subsequently divided into categories based on the each 10<sup>th</sup> centile. Each centile group was given a corresponding score of 1 to 10 ( $< 10^{\text{th}}=1$ ,  $10^{\text{th}}-20^{\text{th}}=2 \dots < 90^{\text{th}}=10$ ). Higher scores were based on the likelihood of increased absorption and distribution, or decreased metabolism or elimination. Scores were tallied for each individual drug and summed across all parameters. The resulting prioritized list looks quite different (with some overlap) from lists created from previous prioritization exercises. The unique pharmacokinetic nature of this list provides a basis for the development of testable hypotheses. This ADME-based list has been used to identify potential additions to analyte list for environmental sample analysis. Internally, the prioritized list has been used to focus research decisions in the process of validating an ADME read-across model. As part of the cohesive strategy moving forward, ADME prioritization should be included as a tool within the overall prioritization strategy, with the highest testing priority given to drugs which rank highly using multiple prioritization schemes. The contents of this abstract neither constitute nor reflect official US EPA policy.

#### 208 Identification of a novel androgen receptor agonist (or "androgen mimic") of environmental concern: spironolactone

C. LaLone, U.S. EPA; D.L. Villeneuve, U.S. EPA / National Health and Environmental Effects Research Laboratory; M. Kahl, U.S. EPA / National Health and Environmental Effects Research Laboratory; E. Durhan, US EPA / Mid-Continent Ecology Division, US-EPA / Research Chemist; E. Makynen, US EPA / Mid-Continent Ecology Division; K. Jensen, U.S. EPA / National Health and Environmental Effects Research Laboratory; J. Cavallin, U.S. EPA, ORISE / National Health and Environmental Effects Research Laboratory; K. Stevens, M. Severson, US EPA; J. Berninger, Baylor University / Department of Environmental Science; S.Y. Skolness, University of Minnesota / Department of Biochemistry and Molecular Biology; J.E. Tietge, US EPA / Office of Research and Development, National Health and Environmental Effects Research Laboratory, Mid-Continent Ecology Division, U.S. EPA / Mid-Continent Ecology Division; C. Russom, US EPA; G.T. Ankley, US EPA / Office of Research and Development, National Health and Environmental Effects Research Laboratory, Mid-Continent Ecology Division, U.S. EPA / National Health and Environmental Effects Research Laboratory. Spironolactone is a pharmaceutical that acts as an androgen receptor (AR) antagonist in humans to treat certain conditions such as hirsutism, various dermatologic afflictions, and female pattern hair loss. The drug is also used to treat hypertension as a diuretic. With this common usage in humans it is conceivable that spironolactone could enter aquatic environments, and indeed has been detected downstream of a pharmaceutical manufacturer.

Predictive methods to analyze human AR orthology suggest that vertebrates would be highly sensitive to chemicals that target the AR compared to invertebrate species. As a means to assess the predictive nature of molecular target orthology leading to unintended effects, 21-d reproduction studies were conducted with two fish species, *Pimephales promelas* (fathead minnow) and *Oryzias latipes* (Japanese medaka) and the invertebrate *Daphnia magna*. Spironolactone significantly reduced fecundity of medaka and fathead minnows at test concentrations of 50 and 5 µg/L, respectively, whereas *Daphnia* reproduction was not affected at 500 µg/L. Masculinization of females of both fish species was observed at 5 µg/L and 0.5 µg/L as evidenced by development of papillary complexes on medaka and tubercle formation on fathead minnows, respectively. Effects in fish occurred at concentrations below those reported in the environment. These results suggest that species with greater orthology to the human AR are susceptible to spironolactone. Further, this drug has the potential to affect reproduction in fish and should be included in environmental monitoring for contaminants of emerging concern. The contents of this abstract neither constitute nor reflect official US EPA policy.

#### 209 Assessing the ecological impacts of pharmaceuticals on invertebrates: insights from a multigenerational study on a common freshwater gastropod

**T.O. Luna**, Texas Tech University/TIEHH / Environmental Toxicology, Texas Tech University; **C.J. Salice**, Texas Tech University / Environmental Toxicology, Texas Tech University / TIEHH, Texas Tech University / Department of Environmental Toxicology. A number of pharmaceuticals have been detected in North American surface waters and are cause for toxicological concern, as some are known endocrine disruptors (EDs). EDs, in particular, can exert sublethal effects at environmentally relevant exposure concentrations in a wide range of vertebrate test species. Unfortunately, fewer studies have focused on understanding the effects of endocrine disruptors on invertebrates. Molluscs, for example, impact primary production, serve as food for small carnivores and can dominate macroinvertebrate biomass. While there are some studies on effects of pharmaceuticals on molluscs, these are limited to a few species and are generally of short duration even though many species can live a year or more. Our objective was to evaluate the effects of multigenerational exposure to commonly occurring pharmaceuticals on life history traits in the freshwater gastropod, *Physa pomilia*. *P. pomilia* was monitored during two generations of continuous exposure to 17- $\alpha$  ethynylestradiol (EE2; 1.0 µg/l, 0.1 µg/l, 0.01 µg/l, 0.0 µg/l) and fluoxetine (100 µg/l, 1.0 µg/l, 0.01 µg/l and, 0.0 µg/l). EE2 is a synthetic hormone found in birth control and fluoxetine is a selective serotonin reuptake inhibitor used as an antidepressant. EE2 significantly affected time to first reproduction, hatching success, egg mass and total egg production but the second generation showed a stronger adverse response than the first. Similarly, fluoxetine significantly affected total egg masses, total eggs, hatching success, size, time to first reproduction, and mortality but, again, the observed effects were mainly in the second generation. Furthermore, the second-generation snails exposed to fluoxetine did not display a typical, monotonic dose response which has similarly been observed by others and suggests endocrine disrupting activity. Current efforts are focused on understanding how the observed effects translate to potential impacts on the population because, in many cases, effects of pharmaceuticals are stimulatory. This population-level assessment will be conducted using a range of population modeling tools to explore and identify the ecological impacts of pharmaceutical exposures to molluscs. Overall, this research provides additional insight into the effects of pharmaceuticals on freshwater invertebrates and points to the utility of multigenerational toxicity studies.

#### 210 The effect of antimicrobial tetracycline on the different levels of biological organization of *Daphnia magna* in multi-generations

**H. Kim**, Gwangju Institute of Science & Technology / Department of Environmental Science and Engineering, Gwangju Institute of Science & Technology / School of Environmental Science and Engineering; **T. Jeong**, Gwangju Institute of Science and Technology (GIST); **S. Kim**, Gwangju Institute of Science and Technology (GIST) / Environmental Science and Engineering. The objectives of this study were to investigate the effect of tetracycline on the different levels of biological organization of *Daphnia magna* to link between genomic and higher level phenotypic stress responses under multi-generational exposure. A range of studies regarding the effect of antimicrobial compounds on non-target organism has been reported, but it has been limited to the one biological organization level such as organism level response. Little is known about the integrated responses at

different biological organization levels. Moreover, toxicity change caused by multi-generational exposure has been reported in a few studies. Using the microarray study, it is expected that biological processes and interlinked pathways in respond to chronic tetracycline exposure to non-target organism can be identified. The target compound was continuously exposed to *D. magna* over four successive generations at the sublethal concentrations including 0.01-10.0 mg/L. For each generation, life-cycle toxicity test for 21 days in static renewal system was conducted. Reproduction and somatic growth related parameters were monitored for the organism level responses and energy related parameters such as energy reserves and consumption were estimated for sub-organism level responses to target compound. The expression of *D. magna* gene is expected to be influenced by the exposure concentration of tetracycline and generation number. Also, *D. magna* genes responded to chronic tetracycline exposure can also be categorized into several functional processes in metabolism and genetic information processes. In addition, the genetic change might have a significant relationship with other biological organization levels. Finally, the integration of responses at different biological organization levels and their relationship can be evaluated using the results of microarray study and phenotypic stress responses in sub-organism, organism and population level responses.

#### 211 Toxic Effects of Anti-Inflammatory Drugs in Neotropical Fish

**J.L. Ribas**, Federal University of Paraná / Pharmacology; **C. Silva**, L.F. Andrade, G.L. Galvan, Federal University of Paraná; **M.M. Cestari**, Federal University of Paraná / Genetic; **E.d. Trindade**, Federal University of Paraná / Cell Biology; **A.R. Zampronio**, H.C. Silva de Assis, Federal University of Paraná / Pharmacology. Several discarded drugs represent health human risks. The use of toxicological data obtained in cell culture may be useful to predict the effects on organisms such as fish and mammals. This work aimed to evaluate the effects of anti-inflammatory drugs as diclofenac (DIC), ibuprofen (IBU), acetaminophen (ACE) and dexamethasone (DEX) on nitric oxide (NO) production and the genotoxic potential (comet assay) of these drugs in anterior kidney cell cultures and erythrocytes from the freshwater fish *Hoplias malabaricus*. Cells from monocytic lineage were separated from the anterior kidney placed and left to adhere for 24h. After this period, non-adherent cells were removed and the remaining cells were treated with DIC(0.2 -2000µg/L), IBU(0.1 - 1000µg/L), ACE(0.025 - 250µg/L) or DEX(0.03-300µg/L) in the presence or absence of 1ng/mL lipopolysaccharide (LPS, *E. coli* 0111:B4). NO concentration was evaluated after 24h in the supernatant of the cultures and the genotoxicity assay was performed in the resulting control cell monolayer (without LPS). The genotoxic potential of these anti-inflammatory drugs was also evaluated, at the same concentrations, in erythrocytes from peripheral blood diluted 1:10 in saline, before contact with the drugs. Data was analysed by One-Way ANOVA followed by Bonferroni's post-hoc test or Kruskal-Wallis followed by Dunns test. There was a reduction in basal NO concentration at 0.2 to 200µg/L of DIC, while for IBU a significant reduction occurred at 100 and 1000µg/L. ACE and DEX reduced significantly basal NO production in all tested concentrations. DIC, IBU, ACE and DEX inhibited LPS-induced NO production in all concentrations tested. Regarding the genotoxicity, no damage was observed in all the concentrations tested. However, genotoxic damage was found in erythrocytes at the lower concentration of ACE, DIC and IBU (0.025, 0.2 and 0.1µg/L, respectively). For DEX, this damage was only observed at 30µg/L. These results suggest that some xenobiotics such as anti-inflammatory drugs may lead to a reduction in monocytic cells activation even though immunosuppression is not their main characteristic. Additionally, the anti-inflammatory drugs tested can have a potential genotoxic effect at low concentrations, but this activity depends on the tissue evaluated. Financial support: CNPq/CAPES

#### 212 Contaminants of Emerging Concern: Contrasting Effects of Pharmaceutical and Estrogen Exposure on Larval Predator Performance

**D.C. Rearick**, St. Cloud State University; **H. Schoenfuss**, St. Cloud State University / Aquatic Toxicology Laboratory, St. Cloud State University / Department of Biological Sciences WSB-273, St. Cloud State University / Department of Biological Sciences MS-273. Contaminants of emerging concern have been linked to numerous endpoints in adult fish. Few studies have examined exposure during earlier ontogenetic stages, where recruitment to reproductive maturity is greatly attenuated by predation. Contaminant induced impairment to escape performance coupled with considerable predation during the larval stage has the potential to cause rapid population decline for short-lived species such as the fathead minnow (*Pimephales*

*promelas*). The objective of this study was to examine the influence of 17- $\beta$  estradiol (E2) at environmentally relevant concentrations (20 ng/L and 100 ng/L) on fathead minnow escape performance. We used a pharmaceutical mixture to contrast consequences between the two contaminant classes. Two assays quantified larval response for individuals exposed 21-days post-hatch using 50% daily static renewal. To measure variation among innate escape behavior, performance elicited by a vibrational stimulus was captured using a high-speed camera (1000 frames/sec). Video data were digitized (ImageJ) to quantify body length (BL in mm), latency (msec), velocity (BL/msec), and total escape performance (BL/msec). To determine whether any decline in escape performance resulted in greater cohort mortality under predation, a predator (bluegill sunfish – *Lepomis macrochirus*) was introduced into an arena containing both contaminate exposed and control larvae. Predation was permitted until approximately half the prey remained. Treatment groups were randomized for staining using SE-Mark for post-predation larval differentiation. E2 exposure increased latency to response ( $p < 0.0001$ ), decreased velocity ( $p < 0.0001$ ), and diminished total escape response ( $p < 0.0001$ ). Survival was higher for control larvae ( $p < 0.0001$ ) with the mean percent ( $\pm 1$  SD) equal to  $55 \pm 13\%$ . No difference was found between E2 concentrations in either assay (Survival low:  $45 \pm 0.14\%$ ; high:  $44 \pm 0.12\%$ ). In contrast, pharmaceutical exposure had no influence on survival ( $p = 0.3$ ), suggesting variation exists among emerging contaminants of concern. Reduction in larval escape performance and survival is suggestive of a mechanism potentially influential to populations. Population modeling may provide foundational insight to understanding the influence of endocrine active compounds during early ontogenetic stages. Funding provided by LCCMR.

### 213 Dose dependent adverse outcomes correlated with specific genomic profiles in fathead minnows upon low-dose exposures to the pharmaceutical fluoxetine

**R. Klaper**, University of Wisconsin-Milwaukee / School of Freshwater Sciences, University of Wisconsin-Milwaukee / Great Lakes Water Institute; J. Weinberger, University of Wisconsin-Milwaukee / School of Freshwater Sciences; J.P. Crago, Great Lakes WATER Institute, University of Wisconsin-Milwaukee, / Student. Pharmaceuticals and personal care products (PPCP) have been found in surface waters globally but at low concentrations. These compounds are not acutely toxic but as they are designed to be bioactive at small concentrations they could have potential impacts on fish and other organisms. Fluoxetine, a selective serotonin reuptake inhibitor is one of the more commonly found compounds in freshwater systems but the impacts of this compound at low concentrations is poorly understood. Previous studies have shown an impact on feeding at relatively high concentrations but have not tested relevant environmental exposures. We tested the effects of low-level exposures of fluoxetine on the reproduction, behavior, and global gene expression patterns in the brain of the toxicological model species *Pimephales promelas* (fathead minnow) for a 4 week chronic exposure. Our data demonstrate that the impacts of fluoxetine on behavior and reproduction are dose and time dependent. In addition gene expression patterns differ significantly with dose and time and indicate that impacts occur at exposures that are lower than those seen using apical endpoints. Gene expression patterns also indicate the possible mechanisms for the increase in aggressive behavior in early exposure intervals versus longer-term exposures. Fluoxetine concentrations as low as 1  $\mu\text{g/L}$  were found to significantly impact certain behaviors in fathead minnow with the most dramatic changes occurring at 10  $\mu\text{g/L}$ . Gene expression patterns demonstrate a noticeable shift at 1  $\mu\text{g/L}$ . Previous studies have attributed impacts of these compounds to serotonin regulation and potential endocrine hormonal changes, however our research demonstrates that pathways involved with hematopoiesis, endocytosis, cell adhesion, neurogenesis, and plasticity are impacted without significant changes in hormone levels in either males or females at these low exposure levels. This is one of the first studies to examine global gene expression changes associated with chronic low-level exposures to fluoxetine and link these changes to adverse outcome endpoints.

**214 Environmentally-relevant mixture of fluoxetine and 17 $\alpha$ -ethynylestradiol reveals estrogen-like interactive effects in male goldfish** H. Silva de Assis, Federal University of Paraná; J.M. Zamora, University of Ottawa / Department of Biology; W.E. Lado, A.M. Al-Ansari, University of Ottawa; D.B. Simmons, J.P. Sherry, Environmental Canada; J.M. Blais, V.L. Trudeau, University of Ottawa. The antidepressant fluoxetine (FLX) and the major active metabolite norfluoxetine are

released to aquatic systems via sewage-treatment effluents with potential endocrine disrupting effects. The synthetic estrogen, 17 $\alpha$ -ethynylestradiol (EE2), is a biologically persistent analogue of estrogen that is widely used in oral contraceptives and hormone replacement therapy. The presence of EE2 in surface waters is attributed to municipal sewage discharges. To better understand possible interactions between these compounds, male goldfish were exposed to environmentally relevant aquatic concentrations of FLX (0.54  $\mu\text{g/L}$ , nominal) and EE2 (5 ng/L, nominal) alone and in combination for 14 days. Ethanol (1.4 mL/70 L tank) was the solvent carrier and also served as the control. Real-time reverse-transcription polymerase chain reaction was used to assess pharmaceutical effects on gene expression and liquid chromatography tandem mass spectrometry (LC-MS/MS) to analyse the plasma proteome. Peptide intensities were calculated from extracted ion chromatograms, or the sum of the precursor m/z abundance from the MS scans (~ chromatographic peak area). The results showed an increase in hepatic estrogen receptor  $\alpha$  (ER $\alpha$ ) and vitellogenin (VTG) gene expression by 1.9-2.4 fold in the FLX and EE2 groups, but this did not reach statistical significance ( $p > 0.05$ ). In marked contrast, co-exposure upregulated hepatic ER $\alpha$  ( $p < 0.05$ ) and VTG gene expression ( $p < 0.05$ ) by 5.5- and 5.3- fold, respectively. FLX and EE2 alone did not affect ER $\beta$ , but the co-exposure downregulated ER $\beta$  expression by 50% ( $p < 0.05$ ). In the plasma, a total of 750 proteins were matched and validated, and the average percent yield of matched and validated spectra was 7%. Of those valid proteins, 474 were identified as network-eligible molecules. There was a significant increase in the number of proteins expressed in goldfish that were related to endocrine system disorders in the FLX and EE2+FLX treatment ( $p < 0.0001$ , Chi-square). Our study demonstrated that an ecotoxicologically relevant simple mixture of FLX and EE2 produces strong estrogen-like effects in the male goldfish. Funded by NSERC, CWN, and CAPES.

**215 Bioavailability-based techniques: Where are we now?** M. Lydy, Southern Illinois University Carbondale / Fisheries and Illinois Aquaculture Center and Department of Zoology, Southern Illinois University-Carbondale / Fisheries and Illinois Aquaculture; A.D. Harwood, Southern Illinois University / Fisheries and Illinois Aquaculture Center and Department of Zoology. The advantages of bioavailability-based techniques over exhaustive sediment extractions are well understood. While exhaustive chemical extractions provide a conservative estimate of exposure to sediment-associated contaminants, bioavailability-based techniques better assess the true hazard in these systems. Bioavailability-based techniques are broadly separated into two main categories: equilibrium- and desorption-based approaches, which represent the bioavailable and bioaccessible fractions, respectively. Since these methods only measure the fraction of contaminant available to the organism, they are more proportional to bioaccumulation. Several of these techniques are in current use including SPME fibers, SPMDs, Tenax TA, as well as others, and these methods are effective at estimating bioaccumulation for a variety of compounds and species. However, it had been hypothesized that these methods could also be effective for estimating toxicity even for biotransformed compounds. Additional applications for these methods include assessing toxicity in field sediments, evaluating remediation success, and source tracking of compounds. Despite these advancements, there are still several data gaps that need to be addressed such as standardization of methodology, further application of toxicity estimates in risk assessments, and a better understanding of the limitations of specific methods. This presentation will review bioavailability-based techniques and provide an introduction for this session.

**216 Assessing bioavailability of DDT and metabolites in marine sediments using SPME with performance reference compounds** L. Bao, University of California Riverside / Chinese Academy of Sciences, University of California, Riverside / Department of Environmental Sciences; F. Jia, University of California, Riverside / Department of Environmental Science; E.Y. Zeng, Guangzhou Institute of Geochemistry, Chinese Academy of Sciences / State Key Laboratory of Organic Geochemistry; J. Gan, University of California, Riverside / Department of Environmental Science. Solid phase microextraction (SPME) has often been used to estimate the freely dissolved concentrations ( $C_{\text{free}}$ ) of organic chemicals in sediments. A significant limitation in the use of SPME for  $C_{\text{free}}$  measurement is the requirement of equilibrium partitioning that is often difficult to meet for highly hydrophobic organic chemicals such as DDT. A method was developed using SPME with stable isotope-labeled analogues as performance reference compounds (PRCs) to measure  $C_{\text{free}}$  in marine sediments. Six PRCs,  $^{13}\text{C}$ - $\alpha$ , $p'$ -DDE,



$^{13}\text{C}$ -*o,p'*-DDD, *p,p'*-DDE- $\text{d}_8$ , *p,p'*-DDD- $\text{d}_8$ , *o,p'*-DDT- $\text{d}_8$ , and *p,p'*-DDT- $\text{d}_8$ , were impregnated into polydimethylsiloxane (PDMS) fiber before use. The isotropy between absorption and desorption between labeled and non-labeled (native) compounds was validated in a range of sediments. With the use of isotope-labeled analogues as PRCs, reproducible measurements of  $C_{\text{free}}$  were achieved using sampling time less than 1 week, as compared to many weeks often needed for the conventional SPME approach. The method showed significant differences in the availability of DDTs in sediments after amendment of activated carbon and sand. The  $C_{\text{free}}$  values derived with the stable isotope-calibrated SPME were statistically equivalent to those obtained using the conventional SPME protocol. Results from this study clearly demonstrated the feasibility of using PRCs in SPME measurements to greatly shorten sampling time, thus affording much improved flexibility in the use of SPME for bioavailability evaluation.

**217 Passive Equilibrium Sampling of Polychlorinated Biphenyls in Baltic Sea Sediment as a Tool to Study Bioaccumulation** A. Jahnke, Stockholm University / Department of applied environmental science, Stockholm University / Inst for tillampad miljoetenskap, ITM; P. Mayer, Aarhus University / National Environmental Research Institute, Aarhus University / Department of Environmental Science, National Environmental Research Inst. / Environmental Chemistry & Microbiology; M. McLachlan, Stockholm University / Department of Applied Environmental Science (ITM). A passive equilibrium sampling approach using glass jars with  $\mu\text{m}$  thin coatings of the silicone polydimethylsiloxane (PDMS) on the inner walls was validated and applied to sediment samples from a 52 km transect in the Stockholm Archipelago. The PDMS coating is brought into contact with the sediment and analysed after equilibrium between the two phases is established, in order to determine freely dissolved concentrations, chemical activities and fugacities. These chemically defined bioavailability parameters for environmental pollutants in sediment are important since sediment is the dominant exposure medium for bottom-dwelling organisms and therefore is particularly relevant in a bioaccumulation context. The coated glass jar method makes use of three different coating thicknesses (2  $\mu\text{m}$ , 4  $\mu\text{m}$  and 8  $\mu\text{m}$ ), which allows equilibrium between the sample and the PDMS to be confirmed for each sample and analyte (QA/QC), while at the same time verifying the absence of sampling artefacts. From the concentrations of polychlorinated biphenyls (PCBs) in the PDMS, we assessed: i) freely dissolved concentrations in the sediment interstitial porewater ( $C_{\text{Sediment, free}}$ ); ii) the equilibrium status between sediment and water; iii) the equilibrium status between sediment and biota; iv) site-specific sediment/water distribution ratios ( $K_D$ ). The results showed that: i) Stockholm remains to be a source of PCBs to the open Baltic Sea manifested in significantly lower  $C_{\text{Sediment, free}}$  at the outer stations; ii) in autumn/winter, the fugacity in sediment exceeds that in water from monitoring programs by an average factor of 4.0; iii) the fugacity in sediment exceeds that in herring from annual monitoring programs by an average factor of 5.2; iv)  $K_D$  values near Stockholm Harbor are by 0.3 up to 1.7 log units greater than in the outer Baltic Sea, possibly due to the strong anthropogenic impact. Altogether, the coated glass jar approach yields precise sediment data at trace levels ( $C_{\text{Sediment, free}}$  between 1.06 fg/L and 1140 pg/L) with built-in QA/QC that provide valuable insight into multimedia environmental processes: As an example, the equilibration of PDMS as the reference phase with sediment, water and biota can provide new, thermodynamically based understanding of bioaccumulation.

**218 Utilizing Passive Sampling for Rapid Response to Assess Atmospheric Exposure to PAHs Before, During and After the Deepwater Horizon Oil Spill** L.G. Tidwell, S. O'Connell, S. Allan, K. Hobbie, G. Wilson, K.A. Anderson, Oregon State University / Environmental & Molecular Toxicology. Polycyclic aromatic hydrocarbons (PAHs) are present in crude oil and may persist in the environment even after visible evidence is gone. Volatilization of crude oil can be one route of exposure for PAHs. Exposures of PAHs from crude oil spills typically occurs concurrent with the spill or prior to significant weathering of the oil. Acute chemical spills generally initiate a rapid clean up response; however, there is often a significant exposure period prior to elaborate air monitoring equipment setup. Because PSDs do not require elaborate equipment or electricity, we were able to initiate sampling within days of the Deepwater Horizon Oil Spill. We deployed passive sampling devices (PSDs) for air sampling prior to, during and after shoreline oiling. Study locations included sites in Louisiana, Mississippi, Alabama and Florida. PSDs mimic the chemical uptake of biomembranes

by exploiting the fugacities of vapor phase compounds in the atmosphere. Chemical data from PSD extracts can be employed to assess potential exposure, transport, fate and sourcing of emerging and legacy contaminants in the atmosphere. Forty two samples were collected over a 15 month period beginning in May 2010; passive sampling devices were used to monitor the bioavailable concentration of PAHs in air. Prior to shoreline oiling, baseline data was obtained at all the study sites, allowing for direct before and after comparisons of PAH air contamination due to oiling. Samples were analyzed using a method composed of 33 emerging and legacy PAHs. As many as 18 PAHs were quantified in some air PSD extracts. Fluoranthene, pyrene and phenanthrene were the largest contributors to the total vapor phase PAH concentration at our sampling sites. PSDs were also screened for an additional 1,200 contaminants of concern. Analysis of PSDs with this screening method resulted in positive identification of oxygen, sulfonate and nitrogen substituted PAHs, as well as pesticides, and compounds used in industry such as musks and flame retardants. Our study demonstrates the utility of air PSDs to respond to acute chemical spills and collect chemical data sets on a wide range of contaminants in a more rapid fashion than traditional air monitoring technologies. During this study OSU's Superfund Research Program Research Translation and Community Engagement Cores developed resources including workshops, brochures, and web-based information available to the communities impacted by the spill.

**219 In vivo passive sampling of nonpolar organic contaminants in brown trout, *Salmo trutta*** L.J. Allan, Norwegian Institute for Water Research, Norsk Institutt for Vannforskning; K. Hawley, T. Haugen, A. Lillicrap, K. Baek, Norwegian Institute for Water Research. Nonpolar organic contaminant concentrations in biota tissues and organs are often assumed to be in equilibrium. This, to an extent, is the reason why a surrogate measure of contaminant body burden can be obtained from measurements in liver or muscle, normalised to lipid content. Contaminant mass transfer within an organism such as fish is expected to be higher than in water. This is the reason why contaminant concentrations in passive sampling phases immersed for 2-4 days in intact fish muscle tissues are expected to reach equilibrium with that in tissue. Another challenge with measurements of contaminant levels in tissues is the normalisation to lipid content. Lipids may or may not be the only sorption phase and different quantification methods for lipids exist. A relatively novel approach to the measurement of non-polar organic contaminant body burden in brown trout (*Salmo trutta*) was applied here. Small tags made of medical-grade polydimethylsiloxane (PDMS) tubing were inserted in the cavity of brown trout. Fish were caged and re-located to a contaminated area of the River Alna in Oslo (Norway) for up to 1 month. Upon retrieval, chlorinated organics (PCBs) were analysed in the tags and in the various tissues (e.g. muscle, liver). Additional in-tissue sampling with a similar PDMS phase was undertaken for comparison with the tags and with lipid-normalised tissue concentrations. The degree of equilibrium reached was evaluated by comparing tissue-PDMS distribution for native PCBs and labelled-PCBs spiked into the tags prior to caged exposures. Fish exposures were supplemented with PDMS-based passive sampling in the water phase and suspended matter sampling. Data obtained was interpreted further using literature values of lipid-PDMS distribution coefficients.

**220 Application of the DGT-2DDFIS/BLM approach to assess site-specific toxicity risk for water and sediment at a river associated with mining activities** T.T. Huynh, University of Queensland / Centre for Mined Land Rehabilitation; B. Noller, The University of Queensland / Centre for Mined Land Rehabilitation. Determining the bioavailable fractions of contaminants in water and sediment is being recognised as a necessary step to predict the effects of metals on biota and for application in the decision tree for assessment of contaminated water and sediment. Bioavailability of a metal generally refers to the potential entry of metal into the ecological domains via the solution phase. The bioavailability of metals in an aquatic system is commonly assumed to mean the ability to be taken up by and across a biological membrane, or as the amount of accumulated metal or metalloid in water that correlates with the observed toxicity in aquatic biota. In a river system, sediment can act as a sink for contaminants in benthic organisms and can potentially be transferred into the aquatic food chain, thus significantly influencing surface water quality. Understanding the kinetics that control the equilibrium of metals in the sediment and water system is crucial, as it can control the metal resupply to biota. The Diffusive Gradients in Thin Film Technique (DGT) Induced Fluxes in Sediment (2D DIFS) is a dynamic numerical model developed to simulate the

transport and reactions occurring within the DGT device and its deployment medium by incorporating first order exchange of solute between solid and solution phases. The 2D DFIS allows accurate determinations of the distribution ratio ( $K_d$ ); equilibration response times ( $T_C$ ) from the DGT interfacial concentration ( $C_{DGT}$ ) measurements; the ratio ( $R_{diff}$ ) of the mean interfacial concentration due to resupply by diffusion concentration to the bulk concentration and then calculate an effective concentration ( $C_E$ ). This study presents a new approach to provide more realistic risk assessments by determining not only the bioavailability but also the kinetics that control the rate of equilibration between them using the DGT-2DDFIS/BLM approach. A total of 5 sampling sites were selected for water and sediment at a river associated with mining activities. Bulk samples were collected for water/sediment quality assessment concurrently with *in situ* deployment of DGTs. The effective concentration ( $C_E$ ) of copper in the sediment is used in the Biotic Ligand Model (BLM) for site-specific criteria derivation instead of the conventional 0.45  $\mu\text{m}$  filtered copper concentration in water. The results for water and sediment quality criteria predicted by BLM using these two different concentrations of copper will be presented and compared.

#### 221 Bioavailability-based chronic toxicity measurements of benzo(a)pyrene and permethrin to *Chironomus dilutus*

J. Du, Guangzhou Institute of Geochemistry, Chinese Academy of Sciences / State Key Laboratory of Organic Geochemistry; J. You, Guangzhou Institute of Geochemistry, Chinese Academy of Sciences / State Key Laboratory of Organic Geochemistry. Benzo(a)pyrene (BaP, a polycyclic aromatic hydrocarbon) and permethrin (a pyrethroid insecticide) are widespread in aquatic ecosystems and their acute toxicity to benthic organisms have been well documented. However, study on chronic toxicity of those contaminants is limited. In the current study, adverse effects of sediment-associated BaP and permethrin on the whole life cycle of *Chironomus dilutus* were determined. The tests were initiated using the newly hatched (< 24 h old) midge larvae and terminated until the hatch of the second generation. The chronic toxicity endpoints included survival, growth, emergence, and reproduction. The BaP concentration of  $340 \pm 2.53$  and  $109 \pm 2.04$   $\mu\text{g/g}$  organic carbon (OC) in sediment caused 50% mortality in *C. dilutus* (LC50) after 20-d exposure and at the end of test, respectively, whereas the LC50 values of permethrin were about 10 times lower than those of BaP and were  $33.1 \pm 1.52$   $\mu\text{g/g}$  OC and  $23.3 \pm 1.37$   $\mu\text{g/g}$  OC, respectively. Rather, BaP and permethrin affected the growth of *C. dilutus* at lower concentrations with the concentration causing 50% effect (EC50) being  $98.0 \pm 1.99$  and  $5.76 \pm 0.76$   $\mu\text{g/g}$  OC, respectively. The EC50 values for emergence of *C. dilutus* were also recorded ( $52.7 \pm 1.72$  and  $3.87 \pm 0.59$   $\mu\text{g/g}$  OC for BaP and permethrin, respectively). In addition, both contaminants affected the sex ratio of the midges as well as egg production rates and hatchability. Recent studies showed that Tenax extractable concentrations better evaluated bioaccumulation and sediment toxicity of hydrophobic organic contaminants than the whole sediment concentrations because it took bioavailability into account. In the current study, bioavailability of sediment-associated BaP and permethrin were measured using a single point 24-h Tenax extraction, and results indicated only about half of the contaminants in sediment were bioaccessible after aging for one month. At last, the response spectra of both contaminants were developed using both OC-normalized whole sediment concentrations and bioavailability-based Tenax extractable concentrations. The development of bioavailability-based chronic toxicity endpoints of sediment-associated BaP and permethrin provide valuable benchmarks for evaluating ecological risk of those contaminants.

#### 222 Applications and advantages of Tenax TA for assessing bioavailability of sediment-associated contaminants

A.D. Harwood, Southern Illinois University / Fisheries and Illinois Aquaculture Center and Department of Zoology; M. Lydy, Fisheries and Illinois Aquaculture Center and Department of Zoology. Tenax TA is a porous polymer resin that has been used to measure desorption rates and quantify the rapidly desorbing fraction of hydrophobic organics from sediments. Studies have shown that both the rapidly desorbing fraction and a single extraction time correlated to bioaccumulation for hydrophobic organics in several species. Unlike other passive samplers, Tenax measures the desorption rates from sediments and is, therefore, a depletive method. It is for this reason, the applicability of Tenax to estimate bioaccumulation and toxicity consistency has been questioned. However, theoretically the fraction measured by equilibrium-based methods, such as SPME fibers, should also be primarily driven by the desorption rates of contaminants from sediments. Therefore, Tenax extractable

concentrations should be proportional to equilibrium-based methods for estimating bioavailability and we have found a correlation between the two measurements. Tenax has been used to successfully estimate bioaccumulation for several species in both laboratory and field-collected sediments for compounds including PCBs, PAHs, and pesticides. Recently, Tenax has demonstrated the potential to estimate toxicity, provide insight to remediation success, and be used for source tracking. There are several advantages to desorption-based techniques, including the rate at which data can be collected and increased sensitivity. Therefore, it is important to recognize this technique and encourage additional research using Tenax.

#### 223 Application of molecular target homology-based approaches to predict species sensitivities to two pesticides, permethrin and propiconazole

C. LaLone, U.S. EPA; D.L. Villeneuve, U.S. EPA / National Health and Environmental Effects Research Laboratory, U.S. EPA; L. Burgoon, C. Russom, US EPA; J. Berninger, Baylor University / Department of Environmental Science; J.E. Tietge, US EPA / Office of Research and Development, National Health and Environmental Effects Research Laboratory, Mid-Continent Ecology Division, U.S. EPA / Mid-Continent Ecology Division; H. Helgen, Computer Sciences Corporation; M. Severson, US EPA; J. Cavallin, U.S. EPA, ORISE / National Health and Environmental Effects Research Laboratory; G.T. Ankley, US EPA / Office of Research and Development, National Health and Environmental Effects Research Laboratory, Mid-Continent Ecology Division. In the U.S., registration of pesticide active ingredients requires a battery of intensive and costly *in vivo* toxicity tests which utilize large numbers of test animals. These tests use a limited array of model species from various aquatic and terrestrial taxa to represent all plants and animals potentially at risk. Predictive methods that systematically and quantitatively assess molecular target homology across species, i.e., at the molecular initiating event level of an adverse outcome pathway, show promise for identifying and ranking species most likely to respond to chemical perturbations of these protein targets. The advent and refinement of these strategies could lead to more focused and integrated approaches to testing and assessment, utilizing the most relevant species with mode of action (MOA) specific toxicity tests, thereby reducing cost and animal use. To further understand and demonstrate the capabilities of protein homology-based species sensitivity predictions, we conducted case studies with two pesticides with known MOAs: permethrin a common insecticide that targets voltage gated para-like sodium channels, and propiconazole a fungicide that inhibits sterol 14  $\alpha$ -demethylase (CYP51). Primary amino acid sequence and conserved functional domain analyses were conducted and non-target species were ranked according to their predicted relative sensitivity to the pesticides. We then compared the results of the homology analyses with empirical toxicity data as a means to demonstrate the appropriate domain(s) of applicability for such predictive methods. This presentation will describe these analyses, and additional progress made in the development of an automated web-tool for such protein-based assessments. The contents of this abstract neither constitute nor reflect official US EPA policy.

#### 224 Multiple ligands derived from pulp and paper mill effluents and trees modulate key molecular receptors and enzymes to suppress reproduction in fish

V.L. Trudeau, University of Ottawa / Advanced Research in Environmental Genomics; A. Waye, Centre for Advanced Research in Environmental Genomics; A. Tang, University of Ottawa / Biology; J.T. Popescu, Centre for Advanced Research in Environmental Genomics / University of Ottawa; J. Arnason, University of Ottawa; M. Hewitt, Environment Canada / National Water Research Institute; C. Milestone, National Water Research Institute; D. MacLachy, Wilfrid Laurier University / Canadian Rivers Institute, Wilfrid Laurier University / Department of Biology; P. Martel, FPInnovations – Paprican Division / Environment; N. Basu, University of Michigan / Department of Environmental Health Sciences, University of Michigan / Department of Environmental Health. Key observations from the Canadian Environmental Effects Monitoring Program indicate that pulp and paper mill effluents (PPMEs) may reduce reproductive potential of fish populations. Initially, it was hypothesized that mill effluents contained substances that interacted with estrogen or androgen receptors. While this is certainly part of the mechanism, it has been difficult to link the cause with the effect. Recent findings demonstrate that some but not all PPMEs could rapidly but reversibly suppress egg production in the fathead minnow. This strongly suggested an alternative hypothesis: that PPME components were affecting the neural (i.e., hypothalamic) control mechanisms of spawning. Bioassay-guided fractionation of PPMEs

from Canada, Brazil and New Zealand, feedstock trees and screening of dozens of known phytochemicals indicate clearly that there are neuroactive substances present. We have found that there are effluent fractions and phytochemicals that can interact with dopamine receptors and also inhibit monoamine oxidase, the key degradation enzyme for dopamine. Other extracts and compounds inhibit the key GABA synthesis enzyme glutamic acid decarboxylase. We performed a large-scale meta-analysis of microarray-derived expression profiles of hypothalamic tissues of PPME-exposed female fathead minnow in comparison with expression profiles of hypothalamic tissues from female goldfish treated with dopaminergic drugs. Effluents that inhibit egg production also affect similar molecular pathways compared with the highly specific dopamine agonists. This is important because it is well-known that dopamine is the single most potent inhibitory regulator of reproduction in teleosts. Components in PPMEs and tree extracts, and pure phytochemicals may increase dopaminergic activity by a combined action on molecular receptors and by inhibition of dopamine degradation. Moreover, it is known that GABA is a potent stimulator of reproduction, so inhibition of GABA synthesis would also contribute to inhibition of reproduction. Collectively, these data support a neuroendocrine disruption hypothesis for the inhibition of reproduction by components in complex PPMEs. Disruption is rapidly initiated at the level of the central nervous system because multiple ligands modulate key molecular receptors and enzymes to suppress reproduction.

**225 Species Differences in Disruption of Androgen Receptor Function by Brominated Flame Retardants** J. Kharlyngdoh, S. Asnake, A. Pradhan, H. Mujahed, Orebro University / School of Science and Technology; P. Olsson, Orebro University / Orebro Life Science Center, Orebro University / School of Science and Technology. With the development of new biological and chemical analyses there is an increased possibility to identify adverse effects of complex and low-level exposures on the health and well being of both humans and wildlife. In contrast to the estrogen receptor that is activated by 17 $\beta$ -estradiol in most vertebrate species, the androgens that activate AR differ between species. While the mammalian AR is preferentially activated by 5 $\alpha$ -dihydrotestosterone, AR from birds and fish are more responsive to testosterone and 11keto-testosterone. This suggests that there may be large species differences in the response to endocrine disrupting compounds (EDCs) with the ability to bind to AR. Using a combination of molecular modeling, receptor activation reporter systems and gene activation assays we are screening for brominated flame-retardants (BFRs) that interact with androgen receptors (AR) from different species. We have earlier identified 1,2-dibromo-4-(ethyl)-cyclohexane (TBECH) as a group of potent agonists to the human AR (hAR). TBECH exist as 4 isomers with corresponding enantiomers. We now have data to show that TBECH also induce AR activation in chicken and zebrafish. However, the responses to TBECH differ substantially between species. In addition to the identification of TBECH we have now identified several additional brominated compounds as androgen antagonists in mammals, birds and fish. Using qPCR arrays we have also obtained results that indicate complex and species specific responses of these androgen antagonists, including regulation of AR transcripts. As some of these BFRs are present in large amounts in the environment, including wildlife, there is a need to further analyze their possible contribution to reproductive disturbances observed in wildlife.

**226 Binding of perfluorochemicals to PPAR $\alpha$ : toward establishing the linkage between the initiating event and its downstream signaling** H. Ishibashi, Shokei University Junior College / Department of Food and Nutrition; M. Hirano, Ehime University / Center for Marine Environmental Studies; E. Kim, Kyung Hee University / Department of Life and Nanopharmaceutical Science and Department of Biology; H. Iwata, Ehime University / Center for Marine Environmental Studies. The peroxisome proliferator-activated receptors  $\alpha$  (PPAR $\alpha$ ) is a member of the ligand-activated nuclear hormone receptor superfamily. It is well known that chronic peroxisome proliferation induced by chemical exposure is associated with the promotion of tumors in the liver of rats and mice. Our previous studies have indicated the induction of hepatic expression levels of PPAR $\alpha$  mRNA and cytochrome P450 4A protein in wild Baikal seals (*Pusa sibirica*) contaminated with perfluorochemicals (PFCs). The *in vitro* reporter gene assay that the Baikal seal PPAR $\alpha$  was transiently expressed in CV-1 cells showed the PPAR $\alpha$ -mediated transactivation by the treatment with PFCs. However, whether a molecular initiating event like the binding of PFCs to PPAR $\alpha$  spans its downstream signaling has not been fully understood. In

this study, we thus measured the binding abilities of PFCs to the Baikal seal PPAR $\alpha$  with the LanthaScreen<sup>TM</sup> TR-FRET PPAR $\alpha$  Competitive Binding Assay. We successfully synthesized the GST-tagged recombinant Baikal seal PPAR $\alpha$ -ligand-binding domain (LBD) using a wheat germ cell-free protein synthesis system. Among eleven PFCs tested, six perfluoroalkyl carboxylates (PFCAs) and two perfluoroalkyl sulfonates (PFSA) bound to the Baikal seal PPAR $\alpha$ -LBD in a dose-dependent manner. PFOA-relative binding affinities (RBAs) of the PFCs were as follows; PFOS > PFDA > PFNA > PFUnDA > PFOA > PFHxS > PFHpA > PFHxA >> PFBA, PFPeA and PFBuS. To our knowledge, this is the first direct evidence of the binding of PFCs to the PPAR $\alpha$ . The quantitative structure-activity relationship analysis showed that PFCAs with 9 or less perfluorinated carbons (PCs) had a positive correlation between the PC number and the RBA of PFCAs, indicating that the PC number is one of the factors determining the binding abilities to the Baikal seal PPAR $\alpha$ . RBAs of PFCs with 7 or less PCs had a positive correlation with the respective PFOA induction equivalency factors (IEFs) obtained by the *in vitro* reporter gene assay. For PFCs with more than 7 PCs, the IEFs were lower than that of PFOA, although the RBAs were higher. These results suggest that this binding assay system may be useful for an initial screening of potential ligands, but the PPAR $\alpha$  binding ability of PFCs may not necessarily be reflected in the transcriptional activity.

**227 In vitro transactivation of avian AHR1 and AHR2 by dioxins to assess the species-specific sensitivity and CYP1A induction in the population-level** H. Iwata, J. Lee, L. Thuruthipallil, Ehime University / Center for Marine Environmental Studies; E. Kim, Kyung Hee University / Department of Life and Nanopharmaceutical Science and Department of Biology. Our recent studies revealed that avian species have at least two isoforms of the aryl hydrocarbon receptor (AHR1 and AHR2). To measure the transactivation potency of cytochrome P450 1A5 (CYP1A5) by TCDD through avian AHR, the reporter construct containing the 5'-flanking region of common cormorant CYP1A5 (*cc*CYP1A5) or chicken CYP1A5 gene (*ck*CYP1A5) was transfected into COS-7 cells with AHR expression plasmid of cormorant (*cc*AHR) or chicken (*ck*AHR). TCDD-EC<sub>50</sub> for the transactivation of *cc*CYP1A5-driven reporter gene by *cc*AHR1 was 0.29 nM, while that of *ck*CYP1A5 by *ck*AHR1 was 0.030 nM. The *cc*AHR1-*cc*CYP1A5 pathway is thus about 10-fold less responsive to TCDD than that of *ck*AHR1-*ck*CYP1A5. In *cc*AHR2-transfected cells, *cc*CYP1A5-reporter gene was dose-dependently transactivated by TCDD. In case of *ck*AHR2, *ck*CYP1A5 construct was constitutively activated, but non-responsive to TCDD. These results suggest that AHR2 contribution to the transactivation may vary among species. Moreover, we examined whether the species difference in the regulatory region of CYP1A5 gene affects the response to TCDD. The measurement of transactivation in a heterologous pair of *cc*AHR1-*ck*CYP1A5 and *ck*AHR1-*cc*CYP1A5 showed that TCDD response was mostly dependent on the species of transfected AHR, not on CYP1A5 construct. To validate whether TCDD dose-response obtained in COS-7 cells corresponds to that in native cells, we treated chicken embryonic hepatocytes with TCDD in which *ck*CYP1A5-reporter construct was transfected. In the hepatocytes, TCDD-EC<sub>50</sub> for *ck*CYP1A5-reporter gene transactivation was 0.046 nM. The similarity of TCDD-EC<sub>50</sub> for *ck*AHR1 in COS-7 with that in chicken hepatocytes suggests that TCDD dose-response in chicken hepatocytes is reproducible in COS-7 cells. As for each AHR isoform from the cormorant and black-footed albatross, TCDD relative potencies (REPs) of dioxin-like congeners (DLCs) were estimated and TCDD induction equivalency factors (IEFs) were determined. Using AHR1- or AHR2-IEFs and hepatic concentrations of DLCs in the respective wild populations, TCDD induction equivalents (IEQs) were calculated. We further constructed non-linear regression models on the relationships between AHR1- or AHR2-IEF derived total IEQs and CYP1A levels in the liver of wild populations. The results suggested that the non-linear regression models were predictable from the *in vitro* assay incorporating AHR of the respective species.

**228 Species- and congener-specific transactivation potencies of red seabream AHR1 and AHR2 by dioxins** S. BAK, Kyung Hee University / Department of Life and Nanopharmaceutical Science and Department of Biology; M. Iida, H. Iwata, Ehime University / Center for Marine Environmental Studies; E. Kim, Kyung Hee University / Department of Life and Nanopharmaceutical Science and Department of Biology. To construct risk assessment system based on aryl hydrocarbon receptor (AHR)-mediated signaling pathway, cross-species comparison of the ligand profile for AHR is



necessary. However, the function of multiple AHRs (AHR1 and AHR2) and the underlying mechanisms of toxicities induced by dioxin like compounds (DLCs) are still poorly understood in fish other than limited model species like zebrafish. Thus we investigated the transactivation potencies of red seabream (*Pagrus major*) AHR1 and AHR2 isoforms (RS-AHR1 and RS-AHR2) by DLCs. To evaluate the transactivation potencies of each AHR by DLCs, we constructed an *in vitro* reporter gene assay system. Each RS-AHR expression plasmid and a reporter plasmid containing red seabream CYP1A (RS-CYP1A) promoter with xenobiotic response elements (XREs) were transfected in COS-7 cells, and the cells were treated with seven selected DLC congeners. Results showed that both RS-AHR1 and AHR2 were dose-dependently activated by all the tested chemicals in this assay. This suggests that AHR1 and AHR2 isoforms play critical roles in CYP1A induction and dioxin toxicities may be triggered by both AHRs in the red seabream. These results of the AHR-mediated transactivation potency are contrastive to those of zebrafish AHR studies; AHR1 produced minimal XRE-driven transactivation but AHR2 induced the transcription in TCDD-treated COS-7 cells. TCDD induction equivalency factors (IEFs) of the tested DLCs for each RS-AHR isoform were estimated from the dose-responses in the reporter gene assay. Comparison of IEFs between RS-AHR1 and RS-AHR2 revealed that PeCDD-IEF for RS-AHR1 is ~10-fold lower than that for RS-AHR2 IEF. This suggests that RS-AHR1 and AHR2 have distinct ligand profiles. When RS-AHR1/2 IEFs are compared with WHO fish toxic equivalent factors (TEFs), both RS-AHR1/2 IEFs are higher for TCDF and PeCDF, but lower for PeCDD. These results were different from those of rainbow trout AHR2 alpha-mediated transactivation assay; PeCDD had a higher IEF, and TCDF and PeCDF had similar IEFs. Taken together, the function of fish AHR is unexpectedly diversified across species and isoform.

**229 Understanding the mechanisms of arsenic adaptation in earthworms – plasticity, epigenetic and genetic adaptation** D. Spurgeon, NERC Centre for Ecology and Hydrology; C. Anderson, Centre for Ecology and Hydrology; P. Kille, J. Andre, J. Morgan, Cardiff University. Mineral extraction is associated with the production of metalliferous soils that can contain highly elevated concentrations of trace elements. Even though highly contaminated, some organisms are able to develop viable population on the contaminated soils. Earthworms are one such group. To understand how earthworm are able to adapt and tolerate highly arsenic (and copper) polluted soils we have utilised a systems biology approach to understand the gene networks that are differentially regulated under arsenic exposure and the association between these networks and endogenous metabolism and internal arsenic speciation. The fully sequenced earthworm *Lumbricus rubellus* was the focus for this work. RNAseq analysis conducted using Illumina technology identified a number of pathways that are affected by arsenic exposure. These related to iron homeostasis, an effect borne out by measured changes in tissue iron concentrations and also the metabolism of amino acids. Metabolomic analysis using <sup>1</sup>H-NMR identified a number of metabolites that changed significantly in response to As exposure. Again amino acids were among the most highly significant of these metabolites. Two amino acids in particular, glycine and cysteine, were identified from this analysis. A link between these metabolites and the production of the metal specific peptide phytochelatin was confirmed through additional LC-MS measurement. With phenotypic data in hand, we next sought to address the mechanisms that determine the survival of earthworms at contaminated sites. Initial AFLP profiling in the taxonomical complex *L. rubellus* species complex identified unique profiles associated with populations inhabiting the most arsenic contaminated soils. Epigenetic changes associated with these exposed population was also found using a methylation sensitive AFLP approach. Whether populations show genetic or epigenetic adaptation as the prevalent mechanism for adaptation depended on the earthworm clade considered. Higher throughput SNP mapping using a RADseq approach is current also being applied to these populations with the potential to link genotype with the changes in phenotype that are known to occur in arsenic exposed earthworms.

**230 Use of mutual information theory to decipher the impacts of multiple stressors from molecular through whole-organism level effects** K.A. Gust, ERDC-EL-EP-P / Environmental Laboratory, US Army, Engineer Research and Development Center, US Army Engineer Research & Development Center, US Army Engineer Research and Development Center, ERDC-EL-EP-P; V. Chaitankar, P. Ghosh, Virginia Commonwealth University; M. Wilbanks, US Army Engineer Research and Development

Center; X. Chen, University of Virginia; C. McFarland, U.S. Army Public Health Command (provisional) / and Preventive Medicine, U.S. Army Public Health Command / Health Effects Research Program, U.S. Army Public Health Command, U.S. Army Center for HPPM; L. Talent, Oklahoma State University; E.J. Perkins, US Army Engineering Research & Development / Environmental Laboratory, US Army ERDC / Environmental Processes & Effect Division, US Army Engineering Research & Development / Environmental Genomics and Genetics Team. The complex nature of multiple stressor effects in biological systems requires new approaches for integrating and drawing inferences from highly diversified data sets. In the present study, we leverage a multiple-stressors investigation in the Western fence lizard (WFL, *Sceloporus occidentalis*) where combinations of climate change and habitat degradation related stressors including malarial parasitism, decreased basic resource (food) availability and exposure to chemical contamination (trinitrotoluene, TNT) were investigated in laboratory bioassays. Resulting datasets from this work include 29 toxicological endpoints ranging from whole organism level effects to blood chemistry parameters for single-stressor and pairwise-stressor exposures. In concert, microarray-based transcript expression datasets were also generated based on these bioassays to provide effects assessment at the molecular and metabolic pathway-level of organization. The data sets were merged and standardized using z-scores and then the popular mutual information theory based algorithm “context likelihood of relatedness” and a new in-house algorithm “R\_REVEAL” were utilized to reverse engineer the network-based relationships among transcription factors, protein-coding genes, protein levels in blood serum and ultimately, whole organism-level effects. An illustrative example of analysis results was identification of high network interconnectivity between genes involved in heme metabolic pathways and blood-hemoglobin levels in TNT exposures. Specifically, expression of genes involved in hemoglobin genesis increased in TNT exposures due to TNT-induced red blood cell RBC lysis and subsequent anemia. Additional components of this network included genes involved in hematopoiesis where gene expression was again increased. The overall response indicates a compensatory response to RBC loss where the molecular machinery of the organism has been engaged to produce new RBCs to mitigate anemia. Additional stressors (food limitation and malarial infection) tended to reduce this overall response. This and several other network-based observations will be presented to illustrate the utility of mutual information theory for providing insights given complex multi-stressor, multi-dimensional datasets.

**231 Spatial distribution of alternative flame retardants in air under GAPS Network: calibration of PUF-disk samplers** S. Lee, E. Sverko, L. Ahrens, Environment Canada; T. Harner, Environment Canada / Atmospheric Science & Technology Directorate, Environment Canada / ASTD; M. Shoeib, L. Jantunen, Environment Canada. This study reports air concentration of alternative non-BDE flame retardants (FRs) at the global scale from samples collected during 2005-2006 at more than 40 sites under the Global Atmospheric Passive Sampling (GAPS) Network. Under the current study, samples collected at GAPS sites over four, three-month consecutive periods between 2005 and 2006, were analyzed for alternative FRs. Samples and field blanks were screened for 16 non-BDE FRs: 1,2-bis(2,4,6-tribromophenoxy)ethane (BTBPE/TBE); allyl 2,4,6-tribromophenyl ether (ATE/TBPAE); 2-bromoallyl 2,4,6-tribromophenyl ether (BATE); 2,3,5,6-tetrabromo-p-xylene (pTBX); pentabromobenzene (PBBe); hexabromobenzene (HBB); tetrabromo-o-chlorotoluene (TBCT); pentabromotoluene (PBTol); pentabromobenzylacrylate (PBBA); 2,3-dibromopropyl-2,4,6-tribromophenyl ether (DPTE); 2-ethylhexyl-2,3,4,5-tetrabromobenzoate (TBB/ EHTEBB); bis(2-ethyl-1-hexyl) tetrabromophthalate (TBPH/BEHTBP); 2,2',4,5,5'-pentabromobiphenyl (BB101); hexabromocyclododecane (HBCD); pentabromoethylbenzene (PBEB); and octabromotrimethylphenylindane (OBIND). Based on concentration data for all sampling periods, BTBPE had the highest frequency of detection (85%) globally, followed by ATE (71%), PBBe (67%), HBB (66%), TBCT (63%), TBB (60%), HBCD (56%), PBEB (49%), TBPH (46%) and DPTE (42%). The remaining non-BDE FRs were detected in 35% or less of the samples. BTBPE concentrations in air ranged from BDL to 187 pg/m<sup>3</sup>. Concentrations (pg/m<sup>3</sup>) for other non-BDE FRs were: ATE, BDL–8.3; PBBe, BDL–2.0; HBB, BDL–5.6; TBCT, BDL–2.8; TBB, BDL–170; HBCD, BDL–220; PBEB, BDL–7.6; TBPH, BDL–66; and DPTE, BDL–12. A field calibration study was undertaken over almost 1 full year to assess the performance of PUF disk samplers for a range of compound classes including high K<sub>ow</sub> FRs that are associated mainly with

the particle-phase. Preliminary results indicate that PUF disk samplers capture both gas-phase and particle-phase FRs with approximately the same sampling rate of 3–5 m<sup>3</sup>/day. These findings show promise for the use of PUF disk samplers for measuring both gas- and particle-associated chemicals in air. The GAPS Network samples are also being analysed for organophosphorous FRs to assess the applicability of PUF disk passive air samplers to these priority chemicals.

**232 Atmospheric distribution of flame retardants in Chicago, Illinois** Y. Ma, Indiana University / School of Public and Environmental Affairs; M. Venier, Indiana University / School of Public and Environmental Affairs, Indiana University; Z. Rodenburg, University of Iowa; D. Hu, The University of Iowa / Department of Civil & Environmental Engineering, IIHR-Hydrosience and Engineering; K.C. Hornbuckle, University of Iowa / Department of Civil & Environmental Engineering, IIHR-Hydrosience and Engineering; R.A. Hites, Indiana University / School of Public & Environmental Affairs,. Flame retardants are added to electronics, textiles, plastics, and furniture foam and are released into the environment as these products are used or discarded. Urban areas have large stocks of products treated with flame retardants, and as a result, people can be exposed to these chemicals through air, water, or dust. Here, we focus on the air of the greater Chicago, Illinois, area. We report the atmospheric concentrations of organohalogen and organophosphorus flame retardants [including, the polybrominated diphenyl ethers, 2-ethylhexyl-2,3,4,5-tetrabromobenzoate, *bis*(2-ethylhexyl) tetrabromophthalate, triphenyl phosphate, and *tris*(1,3-dichloro-2-propyl) phosphate]. Samples were collected along an urban-rural transect through downtown Chicago and along an urban-lake transect extending out to two water intake cribs located about 5 km east of the Lake Michigan shoreline. Passive samplers equipped with polyurethane foam absorbents have been deployed at each site every six weeks since December 2010. This study is designed to analyze the spatial variance of the flame retardant concentrations among the sampling sites and to estimate the possible source contributions of the urban area of Chicago to Lake Michigan.

**233 Heterogeneous Reactions of O<sub>3</sub>, OH Radicals and N<sub>2</sub>O<sub>5</sub> with Particulate-associated PAHs and Toxicity** N. Jariyasopit, Oregon State University / Department of Environmental & Molecular Toxicology; K. Zimmermann, University of California, Riverside / Air Pollution Research Center; J. Schrlau, Oregon State University / Environmental and Molecular Toxicology; J. Arey, R. Atkinson, University of California, Riverside / Air Pollution Research Center; S. Simonich, Oregon State University / Depts. of Chemistry and Environmental & Molecular Toxicology. In the atmosphere, polycyclic aromatic hydrocarbons (PAHs) partition between the gas and particulate phases depending on their chemical and physical properties and ambient temperature. In general, PAHs with more than four aromatic rings are found primarily in the particulate phase, allowing them to undergo long range atmospheric transport. The reactivity of particulate-bound PAHs is considered to vary, to some extent, with the composition of the particles. Previous heterogeneous reaction studies on particulate matter (PM) have used graphite, diesel soot, wood smoke and in-house soot samples. However, in this study, PM from Beijing, China was exposed to O<sub>3</sub>, OH radicals, and N<sub>2</sub>O<sub>5</sub> to simulate heterogeneous reactions that may occur during trans-Pacific transport. The PM samples were collected over 24 h periods at an urban site in Beijing, China. The filters were cut into two portions. The first portion was quantitatively analyzed for PAHs, nitrated-PAHs (NPAHs) and oxygenated-PAHs (OPAHs) and represented the unreacted masses. The second portion was placed in a ~7000-L indoor all-Teflon chamber and exposed to either O<sub>3</sub>, OH radicals, or N<sub>2</sub>O<sub>5</sub>. The concentrations of O<sub>3</sub>, OH radicals, and N<sub>2</sub>O<sub>5</sub> used in chamber experiments, combined with the duration of the exposures, simulated trans-Pacific atmospheric transport of the PM. The quantitative analysis of both the unreacted and reacted portions of the filter was done using gas chromatography coupled with mass spectrometry, with electron ionization for PAHs and negative chemical ionization mode for NPAHs and OPAHs. Analogous to the chemical studies, toxicological studies were carried out on the unreacted and reacted filters. Direct- and indirect-acting mutagenicities of the extracts were determined by Ames assays. In addition, PM samples were characterized by black carbon (BC) and organic carbon (OC) analyses. Our initial results show evidence of the occurrence of heterogeneous reactions of PAHs with the oxidants. N<sub>2</sub>O<sub>5</sub> was the most effective oxidant in transforming the particulate-bound PAHs. The concentrations of 1-nitropyrene, 1-nitrotriphenylene, 6-nitrochrysene, 2-nitrotriphenylene and 6-nitrobenzo[a]

pyrene increased significantly, while the concentration of 9-nitroanthracene decreased significantly. For the OH-radical exposure, only 1-nitropyrene and 6-nitrobenzo[a]pyrene concentrations were found to increase significantly. As expected, no significant NPAH products were formed in the O<sub>3</sub> reaction.

**234 Influence of future emissions and climate on atmospheric PAH transport** C. Friedman, MIT / Center for Global Change Science, University of Rhode Island; N.E. Selin, Massachusetts Institute of Technology / Engineering Systems Division. We use the 3D atmospheric chemical transport model GEOS-Chem to investigate the impacts of global changes, including emissions and climate, on long-range polycyclic aromatic hydrocarbon (PAH) transport. Unlike most persistent organic pollutants (POPs), PAHs are emitted unintentionally as byproducts of combustion, and their release to the atmosphere is ongoing. In the Arctic, PAHs have been termed “emerging contaminants” because marine animal tissue concentrations are increasing while other POP concentrations are declining. We assess the influence of global anthropogenic emissions changes and climate change on the transport of phenanthrene (PHE), pyrene (PYR), and benzo[a]pyrene (BaP) to the Arctic, focusing on categorizing the relative importance of these global changes. We conduct simulations with GEOS-Chem varying emissions and climate respectively. We estimate future PAH emissions for global regions based on expected changes in gross domestic product and average income, and also assess potential Arctic emission increases due to greater oil/gas exploration and shipping activity. Climate variables assessed include higher global mean temperatures, shifts in atmospheric circulation patterns, and changes in natural PAH emissions, such as from wildfires. We find that climate changes will reduce transport of primary emitted BaP via increases in wet deposition of the particle phase, but will have less impact on PHE and PYR. Emission reductions in developed regions will likely have a greater impact in the Arctic compared to increases in developing regions because of their spatial proximity.

**235 Atmospheric deposition of PCBs to the Delaware River** L.A. Rodenburg, Rutgers, the State University of New Jersey / Department of Environmental Sciences, RUTGERS UNIVERSITY / Department of Environmental Sciences; J. Guo, Rutgers, the State University of New Jersey / Department of Environmental Science, Rutgers Univ. / Environmental Science; S. Du, RUTGERS UNIVERSITY / Department of Environmental Science; T. Fikslin, G.J. Cavallo, Delaware River Basin Commission. In 2003, the Delaware River Basin Commission (DRBC) promulgated a Total Maximum Daily Load (TMDL) for Polychlorinated Biphenyls (PCBs) in zones 2 through 5 of the Delaware River. This was one of the first TMDLs for a persistent organic pollutant in a major US waterway. In support of the TMDL promulgation and implementation, the DRBC funded the Delaware Atmospheric Deposition Network (DADN), which grew out of the New Jersey Atmospheric Deposition Network. These two networks operated from 1996 to 2011 and comprised a total of 13 sites, although not all sites operated in all years. PCBs were measured in the gas, aerosol, and precipitation phases, and the resulting concentrations were used to calculate the deposition of PCBs to the Delaware River. In addition to the fixed monitoring sites, the DADN project also included two passive sampling campaigns designed to assess the spatial heterogeneity in gas-phase PCB concentrations. The data gathered by the DADN project has been used to investigate urban PCB sources, to examine the extent to which watersheds sequester their atmospheric PCB loads, and to better integrate water quality models with atmospheric data.

**236 Modeling urban areas as a source of PCBs and PBDEs to surrounding regions using a coupled multimedia and atmospheric transport model** S.A. Csizsar, University of Toronto / Dept. of Chemical Engineering and Applied Chemistry, , University of Toronto; L. Melymuk, University of Toronto / Dept. of Chemical Engineering and Applied Chemistry; S.M. Daggupati, Environment Canada; P.A. Helm, Ontario Ministry of the Environment / Environmental Monitoring and Reporting Branch; M.L. Diamond, University of Toronto / Department of Geography and program in planning, University of Toronto / Department of Geography. Urban areas, with their elevated persistent organic pollutant (POP) air concentrations as well as high geographic concentration of chemical inventories, are sources of polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) to surrounding regions. We estimated spatially resolved (on a 25km<sup>2</sup> scale) Σ<sub>88</sub>PCB and Σ<sub>26</sub>PBDE emissions to be ~140 and ~30 kg/y, respectively, based on chemical mass inventories and measured air data



from Toronto, Canada which borders Lake Ontario. Using these emission estimates and the coupled Multimedia Urban Model and the Boundary Layer Forecast Model (BLFM), called SO-MUM, we estimated the urban fate pathways and the importance of primary versus secondary emissions of PCBs and PBDEs by modeling reduced emission scenarios. SO-MUM estimated that ~95% and ~70% of air emissions were advected out of the city for PCBs and PBDEs, respectively. Urban surface films increased the air transport potential of PCBs whereas they acted as a transient sink from air for PBDEs allowing for further mobilization to storm water. The urban air plume traveled 60 and 50 km for PCBs and PBDEs, respectively, where concentrations dropped to background levels. We call this distance the Urban Travel Distance. However, the distances to reach 1/e or 63% were 25 and 30 km for PCBs and PBDEs, respectively. We suggest that the greater extent of the PBDE plume using 1/e estimate than 1/10 is due to the broader geographic distribution of the PBDE versus PCB inventory since PBDEs travel distances from source to sink are shorter than PCBs based on physical-chemical properties. For a spring 2008 simulation, the model predicted that Lake Ontario was a source to air for PCBs and a sink from air for PBDEs. The main urban loading pathway to Lake Ontario was wash-off from soils (~90%) for PCBs, and for PBDEs approximately equally air deposition (~56%) and wash-off from surface films (~39%) to storm waters. When the PCB inventory and emissions are reduced to less than 10 times, Lake Ontario becomes the main source of PCBs to air (secondary emissions), however at levels ~3 times lower than current estimated primary emissions. Secondary emissions of PBDEs to air from Lake Ontario or soils are expected to be negligible even at reductions of the inventory and primary emissions by 1000 times.

**237 Metals, PCBs, PAHs, Dioxins and Furans in ambient dust and rainfall along an urban-rural watershed gradient** R. Jack, King County / Department of Natural Resources; J.A. Colton, King County Department of Natural Resources / Water and Land Resources Division, King Cty. Dept. of Nat. Resources / Water and Land Resources Division. This talk presents project information along with sampling and analytical methodologies for a year-long study of atmospheric deposition in the Green/Duwamish River Basin in western Washington State, USA. The objective of this study is to compare the measurements of bulk deposition (combined dry particulate and rainfall) at a small number of stations in areas of different land use within the Green/Duwamish River Basin. These measurements provide the first atmospheric deposition data for many of these chemicals covering a western Washington basin. The goal of this study is to understand atmospheric sources to the Lower Duwamish Waterway (LDW) a 5-mile long, working harbor and Superfund site. Samples were analyzed for select metals, mercury, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), seven polychlorinated dibenzo-dioxins (PCDDs), and ten polychlorinated dibenzo-furans (PCDFs). These chemicals have been identified as contaminants of concern (COCs) for the LDW Superfund site. The sampling method was consistent with that used in previous King County and Puget Sound Partnership air deposition studies. Data collected from this study enhance the understanding of how atmospheric deposition may contribute COCs to the combined sewer system leading to the LDW in Seattle, Washington. The data also allow estimation of bulk deposition from upper watershed areas within the Duwamish/Green River Basin which contributes COCs to the Green River, and subsequently the LDW.

**238 Sources of Aroclor and Non-Aroclor PCB congeners in urban, industrial, rural and indoor environments** K.C. Hornbuckle, University of Iowa / Department of Civil & Environmental Engineering, IIHR-Hydroscience and Engineering. Polychlorinated biphenyls (PCBs) are found in ambient air throughout the world and are an important source of PCBs in fish and other wildlife. PCBs have both historical (Aroclors and other commercial mixtures) and current (manufacturing byproducts) origins. PCB congeners of all molecular weights are found in the gas phase but the sources of gas-phase PCBs are not well understood, even in highly contaminated cities and industrial regions. The major categories of gas-phase PCB sources include: 1) Volatilization from soils historically contaminated from use and disposal of commercial mixtures; 2) Volatilization from contaminated waters and underlying sediments; 3) Volatilization from PCB-containing building materials; 4) Emission of non-Aroclor congeners as by-products of manufacturing and; 5) Emission to ambient air from contaminated indoor air. We have conducted field studies of airborne PCB congeners in urban and rural environments and have found evidence for localized sources of each of these

categories. Here we summarize our findings about residential soils in a small midwestern city; emissions from contaminated sediments of an industrial harbor; the presence of PCBs in current commercial house paint; and emission of PCBs from contaminated indoor air ventilation. The nature of these sources is identified primarily through direct measurements of a large suite of congeners (up to 170 congener groups and all 209 congeners) using tandem mass spectrometry. These analytical techniques make analysis of congener patterns a useful component of mechanistic models that describe PCB emissions from distinct source categories.

**239 A Different Type of Urban Runoff Management Practice – Pollution Prevention** K.D. Moran, TDC Environmental, LLC. Research on urban runoff treatment systems has shown that some common toxic pollutants—like copper and pesticides—cannot be effectively removed by compact treatment systems (like storm drain inserts) that are readily installed in urban areas. It is unlikely that existing cities will ever be fully retrofitted to treat all of the nation's urban runoff with the types of land-intensive treatment systems capable of achieving water quality standards, due to land cost and unavailability, the costs for drainage infrastructure reconstruction, and the associated societal disruption. Land-intensive treatment is not the only option. Another management practice can control hard-to-treat pollutants—Pollution Prevention. Pollution Prevention involves two general strategies: (1) modifying how pollutant-containing products are used and (2) product reformulation. Examples of Pollution Prevention include the elimination of lead from vehicle fuels, phase out of most diazinon and chlorpyrifos use in urban areas, local ordinances prohibiting use of coal tar pavement sealcoat, California regulations limiting how pyrethroid insecticides are applied by professional structural pest control applicators, and vehicle brake pad reformulation to remove lead and copper. Unlike treatment systems, effective pollution prevention control strategies that do not have undesirable side effects are not generic. They must be custom designed for each pollutant based on research, starting with a pollutant source identification study that identifies the true (product) source(s) of the pollutant. Follow-up research depends on the pollutant's sources, but often includes development of robust data linking pollutant-containing products to pollutant loads in urban runoff, product usage characterization, and examination of the feasibility and potential environmental consequences of potential control strategies. The presentation will review specific examples of the research behind the design and implementation of Pollution Prevention control strategies for pesticides and metals in urban runoff, discuss the strengths and shortcomings of the measures that were implemented, and summarize available data characterizing effectiveness in reducing pollutant loads.

**240 Evaluating the effects on lake biota of chronic exposure to two P-inactivation agents and providing lake managers with a context for the results** S.J. Clearwater, National Institute of Water & Atmospheric Research / NIWA; C.W. Hickey, K.A. Thompson, National Institute of Water and Atmospheric Research. Application of phosphorus (P) inactivation agents is a eutrophic lake management technique to reduce the internal release of P from lake sediments. Effective mitigation of nutrient release from lake sediments may require the repeated application of large quantities (i.e., tonnes) of P-inactivation agents, therefore rigorous evaluation of non-target effects as well as efficacy is essential. Both acute and chronic effects must be evaluated, using both field and laboratory exposures. A laboratory mesocosm approach was used to evaluate the effects of chronic exposure (two months) to alum (aluminium sulphate solution buffered with sodium carbonate; 3 application rates 37, 73, or 220 g alum m<sup>-2</sup>; n = 3) or 200 g m<sup>-2</sup> Aqual-P<sup>TM</sup> (a proprietary product based on aluminium-amended zeolite) on freshwater crayfish *Paraneoprops planifrons*, fingernail clams *Sphaerium novaezelandiae*, mussels *Echyridella menziesii* and fish *Gobiomorphus cotidianus*. Combining the species in a flow-through mesocosm incorporates the effects of bioturbation, especially by the crayfish, on the inactivation agents. Site-specific information was provided by using sediments from the application site (Lake Rotorua, central North Island, New Zealand). In comparison to the control treatment, growth and survival of the fish, mussels and crayfish was not significantly affected by two months exposure to the P-inactivation agents, but clam survival, and reburial rates decreased significantly after exposure to the highest alum dose ( $P < 0.001$ ). Oxygen consumption rates were slightly lower in mussels exposed to Aqual-P ( $P < 0.04$ ), otherwise oxygen consumption rates and ammonia excretion by the mussels and crayfish were unaffected by the treatments. Crayfish accumulated aluminium in the hepatopancreas in a dose-dependent response to alum exposure ( $P < 0.001$ ),



but not after the exposure to Aqual-P. These results establish a threshold alum dose of  $73 \text{ g m}^{-2}$ , or  $200 \text{ g m}^{-2}$  Aqual-P for effects on these non-target species. These application rates will be put into context with factors such as the proposed application to the deep, anoxic zone of the lake where few benthic macroorganisms are present, and product characteristics such as different P-binding rates, metal- or ammonia-binding, or metal loss rates, and effects on the sediment microbial community resulting in ammonia release.

**241 Evaluation of a Vegetated Treatment System and Landguard A900 Enzyme: Reduction of Water Toxicity Caused by Organophosphate and Pyrethroid Pesticides** B.M. Phillips, University of California – Davis / Environmental Toxicology, University of California-Davis / Granite Canyon; B. Anderson, UC Davis / Environmental Toxicology; K. Siegler, J. Voorhees, University of California Davis / Environmental Toxicology; R. Tjeerdema, UC Davis / Environmental Toxicology; R. Shihadeh, P. Robins, Monterey County Resource Conservation District; R. Budd, California Department of Pesticide Regulation. Runoff from irrigated agriculture in Monterey County contributes a significant amount of water to local stream flow, and several studies have measured toxic pesticide concentrations and biological impacts in receiving systems. On-farm practices such as vegetated treatment systems (VTS) and enzyme application can reduce concentrations of pesticides in runoff. A redesigned integrated VTS was evaluated with a series of field experiments. The VTS was constructed in a ditch that included a 40m section for sedimentation, a 170m section of vegetation, and included a flashboard riser to control the volume of water in the vegetated section. Laboratory experiments were conducted to determine the optimal dose and mixing time of Landguard A900 enzyme to reduce concentrations of organophosphate pesticides. A series of trials were conducted on a larger, unvegetated drainage ditch to determine the efficacy of the enzyme in a setting with up to twenty times the discharge volume. Field trials included measurements of water toxicity and chemistry at the input and output of each system. These trials were conducted during actual irrigation events that varied in runoff magnitude. The VTS reduced concentrations of pyrethroids, organochlorines and total suspended solids by 97-100%. Landguard application in the larger drainage completely removed chlorpyrifos and diazinon.

**242 Overview of Urban and Agricultural Stormwater Treatment Projects** N. David, D. Yee, San Francisco Estuary Institute; L.J. McKee, San Francisco Estuary Institute / Watershed Program. Detrimental impacts of stormwater runoff from transportation infrastructure and intensive agricultural land use have been well documented. The performance of a variety of Best Management Practices (BMPs) and Low Impact Development practices (LID) for reducing pollutant loads from stormwater is being monitored in northern California. In urban settings, reductions can be achieved through the construction of rain gardens, vegetated swales, infiltration and flow-through planters, curbside extensions, or a combination of these techniques, and structural BMPs in drainages (e.g., filters), coupled with monitoring to evaluate their effectiveness. Additionally, walnut and stone fruit orchard runoff was monitored in the Central Valley of California to examine growing practices such as integrated pest management, and certified organic practices. The agricultural BMPs implemented included the use of organic pesticides and pheromone disruption, cover crops, filter strips, beneficial insects, and monitoring of insects and insect fertility. The results from these studies suggest that rain gardens and swales are effective in reducing pollutant loads from urban stormwater runoff. They also suggest that pesticide and nutrient loads can be reduced in agricultural runoff during irrigation and storms. Pollutants that bind strongly to sediment particles (e.g., most metals, PCBs, PAH, pyrethroids) are especially amenable to removal relative to dissolved phase pollutants when flow is reduced and stormwater is filtered. Despite these differences in treatment efficiency, depending on the physical characteristics of the pollutant and the treatment method, the data indicate reductions in pollutant concentrations and loads between 40 and 90% even in larger storm events.

**243 Phytoremediation of atrazine-contaminated water by expression of anti-atrazine antibody fragment (scFv) in duckweed (*Lemna minor*)** S. Leelachao, University of Guelph / School of Environmental Sciences; A. Ziauddin, K.R. Solomon, University of Guelph; J.M. Hall, Arkansas State University / Department of Mathematics and Statistics. Atrazine is widely used as the main active ingredient for broadleaf weed control in corn, sugarcane and sorghum crops. Atrazine is an environmental concern since water resources may be contaminated with atrazine from runoff emanating from

corn and sorghum fields. An anti-atrazine single chain variable fragment (scFv) gene was randomly inserted into the genome of common duckweed (*L. minor*), an aquatic macrophyte, to sequester atrazine in the transformed plant. Transgenic *L. minor* was produced via *Agrobacterium*-mediated transformation. The molecular weight and expression of the poly-histidine tagged scFv with was confirmed by Western blot. It was determined using dose-response analysis ( $EC_{50}$ ) that the wild-type versus transgenic *L. minor* was more sensitive to atrazine 7 days after exposure.  $EC_{50}$  values for wild-type and transgenic *L. minor* were significantly different. The results of this proof of concept study suggest that antibody expression in plants can potentially be used for phytoremediation of waters contaminated with atrazine.

**244 Potential remediation of nitrate-contaminated water in space-limited areas using microbial-based bioreactors** P. Wilson, University of Florida / IFAS / IRREC- Soil & Water Science; J. Albano, USDA/ARS-Horticulture Research Laboratory. Nitrate is one of the most common contaminants in surface water throughout the world. Losses in surface runoff and drainage water from nursery and agricultural production areas, as well as from the managed landscapes can be significant. This study evaluated the potential use of microbial-based (denitrification), flow-through bioreactors for their nitrate-remediation ability. Duplicate bioreactor systems were constructed at a local foliage plant nursery. Each bioreactor system consisted of four 242 L tanks with connections alternating between bottom and top. Each tank was filled with approximately 113 L of Kaldness media to provide surface area for attachment of native microflora. Molasses was supplied as a carbon source for denitrification and water flow rates through the systems ranged from 5 to  $18 \text{ L} \cdot \text{min}^{-1}$  during tests. Automatic water samplers were used to collect composite samples every 15 minutes from both the inflow and the exit flow water. Results indicate consistent removal of 80-100% of the nitrate flowing into the systems. Accumulation of ammoniacal and nitrite nitrogen did not occur, indicating that the nitrate-nitrogen was removed from the water, and not simply transformed into another water-soluble species. Occasions where removal rates were less than 80% were usually traced to faulty delivery of the carbon source. Results indicate that modular microbial-based bioremediation systems may be a useful tool for helping water managers meet stringent nitrogen water quality regulations, especially in areas with limited space for construction or expansion of water retention facilities.

**245 The potential of using rice (*Oryza sativa*) to mitigate agricultural runoff** M.T. Moore, USDA-ARS, National Sedimentation Laboratory, USDA-ARS National Sedimentation Laboratory / National Sedimentation Laboratory; M.A. Locke, USDA-ARS National Sedimentation Laboratory / National Sedimentation Laboratory. Agriculture is faced with providing food and fiber for a growing global population. With little new land availability, farmers must maximize production on limited acreage. Often these challenges are intersected by precipitation events which lead to non-point source runoff discharges. Innovative, cost-effective conservation practices are needed to address issues of agricultural runoff mitigation. A current approach being examined is the management of existing rice (*Oryza sativa*) fields for phytoremediation and mitigation of pesticides and nutrients. Scaled experiments studied the efficiency of rice at mitigating concentrations and loads of various pesticides. Initial results are promising, with rice mitigating 71-92% of applied pesticide mass, while non-vegetated systems removed only 42-51% of pesticide mass. Additional studies are examining possible pesticide transfer from plant to seed to determine if rice can also serve as a food source for developing countries' efforts at water quality improvement.

**246 Using Groundwater Protectiveness Demonstration Tools to incorporate more UICs into Stormwater Management Plans** H.H. Blischke, M. Kohlbecker, GSI Water Solutions, Inc.; B. Adkins, City of Portland / Bureau of Environmental Services. Stormwater is widely viewed as a source of contamination to surface water. Concentrations of copper, biological oxygen demand (BOD), and temperature that are highly detrimental to ecological receptors in surface water are readily attenuated in the natural treatment capabilities of the subsurface environment. Underground Injection Control Devices (UICs) are included in the City of Portland's comprehensive watershed plan to use stormwater as a resource by infiltrating it back into the ground. UICs preclude the need to install or increase the capacity of piped stormwater infrastructure that eventually discharges into local surface water bodies. In 2005, the City of Portland was issued the first

Water Pollution Control Facility (WPCF) permit for UICs in the nation for management of stormwater from public rights-of-way. Permit conditions would have required hundreds of the City's UICs to be decommissioned. Stormwater sampling data from 7 years of monitoring for a wide range of constituents including total and dissolved metals, volatile and semi-volatile organic compounds, and selected herbicides and pesticides collected from over 75 unique UICs showed that pentachlorophenol (PCP) was regularly measured at concentrations above the permit discharge limit of 1 µg/L. Because of its low sorptive properties, PCP was the primary driver posing risk to groundwater. Fate and transport modeling demonstrated that PCP discharged directly into groundwater attenuates to protective concentrations within less than 275 feet of a typical UIC. Vadose zone modeling showed that the chemicals found in municipal stormwater attenuate within 5 feet of the bottom of the UICs. Oregon Department of Environmental Quality (DEQ) concurred with the modeling results allowing the City to retain over 300 UICs that were considered noncompliant under the permit. Groundwater protectiveness demonstrations were successfully conducted for other Oregon municipalities. As a result, DEQ revised the WPCF permit template encouraging the use of UICs to manage stormwater where they can be shown to be protective of groundwater receptors. Use of groundwater protectiveness tools allow for the use of UICs in municipal watershed management plans as a cost-effective method to recharge groundwater, cool water, and attenuate pollutants prior to reaching surface water bodies.

**247 Chemical speciation of dissolved trace metals in estuarine, coastal and open ocean waters: modelling based on humic type ligands** A. Stockdale, Centre for Ecology and Hydrology / Lancaster Environment Centre, University of Manchester / School of Chemistry; E. Tipping, Centre for Ecology & Hydrology; S. Lofts, NERC Centre for Ecology & Hydrology / Shore Section; J. Hamilton-Taylor, Lancaster University. If water quality standards for estuaries and coastal areas are to be formulated on a site specific basis and based on chemical speciation concepts then there is a pressing need to validate chemical speciation models, which are widely used for terrestrial systems, for saltwater environments. We apply the chemical speciation model WHAM/Model VI to investigate the distribution of metal species (of Fe(III) and the divalent cations of Ni, Cu, Zn, Cd, Hg and Pb) in the water column of estuaries and coastal areas as well as open oceans. In doing this we compare, for the same sites, modelled results with experimentally measured values. The advantage to using a single model for a wide range of data sets, is that it can help to identify whether there are consistent trends in activities obtained using different analytical methods, i.e. relative to a consistent set of predictions. We show that in some cases, speciation of trace metals in brackish and seawater environments may be reasonably predicted using this model with its default parameter set, without any model fitting. Comparisons for several metals and different salinity environments will be presented.

**248 Cu and Pb speciation and toxicity to sea urchin larvae (*Paracentrotus lividus*) in marine samples enriched with dissolved organic matter** P. Sanchez Marin, Institut National de la Recherche Scientifique / Centre Eau Terre Environnement, INRS-ETE / INRS-ETE; J. Lorenzo, R. Beiras, University of Vigo. Dissolved organic matter (DOM) may greatly influence metal speciation in seawater environments, due to its complexation capacity, but the effects of DOM on metal bioavailability in seawater are not yet well known. Previous results using artificial seawater enriched with commercial DOM (as humic and fulvic acids) have shown that DOM decreased Cu toxicity to the larvae of the sea urchin, *Paracentrotus lividus*, in agreement with the decrease in labile Cu concentrations as measured by anodic stripping voltammetry (ASV). However, Pb toxicity to *P. lividus* larvae increased in the presence of humic substances, despite their Pb complexation ability. In order to test if these patterns were also observed in natural samples, Cu and Pb toxicity to *P. lividus* was evaluated in different environmental samples: estuarine seawater, two sediment elutriates and sewage-influenced seawater. Cu and Pb complexation properties were measured by anodic stripping voltammetry (ASV), and the carbon content (DOC) and the optical properties (absorbance and fluorescence) of the coloured DOM fraction were determined. The same series of analysis were performed on the samples subjected to a UV-irradiation treatment in order to oxidize the organic matter present. The results confirmed that Cu toxicity can be predicted according to ASV measurements of labile Cu and that DOC content and fluorescence of the DOM are good predictors of the complexation (and protective) capacity of the water towards Cu. Lead complexation was lower than that of Cu

and not enough to significantly affect Pb toxicity, except for an unexpected enhancement of Pb toxicity observed in some UV-irradiated samples. These results contribute towards our understanding of the effects of DOM on Cu and Pb bioavailability in seawater, but they are limited by the relatively high concentrations of Cu and Pb needed to cause toxicity in short-term studies. Similar studies at lower – more environmentally relevant – metal concentrations are clearly needed.

**249 Influence of salinity and DOC on acute Cu toxicity to the rotifer *Brachionus plicatilis*** C.A. Cooper, T. Tait, J.C. McGeer, Wilfrid Laurier University; R. Santore, HDR/HydroQual; S.D. Smith, Wilfrid Laurier University. Static acute copper (Cu) toxicity tests (48-h LC50) using the euryhaline rotifer *Brachionus plicatilis* were performed to assess the effects of salinity and dissolved organic carbon (DOC). Rotifers were acclimated to 3 different salinities (3, 16 and 30 ppt), exposed to 4 different concentrations of DOC (ranging from zero added DOC to 13.7 mg C L<sup>-1</sup>) and total Cu concentrations ranging from 0 to 390 µg L<sup>-1</sup>. Total Cu was measured using anodic stripping voltammetry (ASV) and free Cu concentrations using an ion selective electrode (ISE). There was a protective effect of salinity when no DOC was added; with the LC50 values being significantly higher at 30 ppt (34.4 µg L<sup>-1</sup>; 95% CI = 28.5–43.1 µg L<sup>-1</sup>), when compared to 3 ppt (13.5 µg L<sup>-1</sup>; 95% CI = 11.7–15.6 µg L<sup>-1</sup>) and 16 ppt (14.6 µg L<sup>-1</sup>; 95% CI = 12.9–16.6 µg L<sup>-1</sup>). DOC complexation significantly reduced Cu toxicity. For all salinities, the protective effect of DOC against Cu toxicity was linear up to concentrations of 5 mg C L<sup>-1</sup>. However, at high concentrations of DOC (13.7 mg C L<sup>-1</sup>) the LC50 values were lower than expected; the degree of protectivity per mg of C decreased as DOC increased. This relationship was more pronounced at higher salinities, indicating the possibility of salt induced colloid formation decreasing the number of available reactive sites within DOC to complex Cu. Taking into account LC50 values, DOC concentrations and salinity, modeling of free Cu concentrations appeared to support salt induced colloid formation at high DOC concentrations, as well as the protective effect of increasing cation concentrations on Cu toxicity. ISE measurements of free Cu were comparable to the modeled data at high DOC concentrations, but not so when both salinity and DOC concentrations were low. The most likely cause of this discrepancy was the weak buffering capacity and resultant poor ISE reliability at low total Cu concentrations. Data from this research will be used in the development of a marine and estuarine biotic ligand model, and ultimately criteria for the protection of organisms in these environments.

**250 The influence of salinity on the acute toxicity of Cd to estuarine killifish: A comparison of Cd, Cu, Zn, and Ni toxicity** G.K. Bielmyer, Valdosta State University / Biology, Valdosta State University / Department of Biology; M. Grosell, University of Miami. The euryhaline killifishes, *Fundulus heteroclitus* and *Kryptolebias marmoratus* inhabit estuaries that rapidly change salinity on both daily and seasonal basis. It is well known that metal toxicity in freshwaters can vary with water chemistry and that metal bioavailability is controlled by ligand interactions and competing ions. However, the characterization of metal toxicity in estuarine and marine systems has been given far less attention. Additionally, current environmental regulations do not account for organism physiology in different salinity waters even though metal sensitivity is likely to change in such environments. More data is needed to better understand mechanisms of metal toxicity so that toxicity can be predicted in estuarine waters and criteria can be derived to protect the organisms inhabiting these environments. In this study, we investigated the effects of changing salinity on acute toxicity of Cd to larval (7–9 d old) *F. heteroclitus* and *K. marmoratus*. The 96-h median lethal concentrations (LC50) for Cd were determined for both fish species at six different salinities. Further, previous studies with Cu, Zn, and Ni are reviewed for comparison. In general, as salinity increased, metal toxicity decreased in both fish species. However, clear differences were apparent on the extent of salinity protection among metals and between fish species, particularly at intermediate salinities. These results could be useful for development of marine/estuarine biotic ligand models and for the determination of site-specific water quality criteria.

**251 Toxic physiological effects of Pb, Zn, Cu and Ni on early life stages of the sea urchin (*Strongylocentrotus purpuratus*)** M.S. Tellis, McMaster University / Biology; M.M. Lauer, Federal University of Rio Grande; S.R. Nadella, McMaster University / Biology; A. Bianchini, Federal University of Rio Grande; C.M. Wood, McMaster University / Department of Biology.

The early life stages of echinoderms are among the most sensitive organisms to many contaminants in the marine environment. In order to elucidate mechanisms of metal toxicity, we examined the physiological responses of larvae of *S. purpuratus* in 100% sea water to continuous sublethal exposure to Zn, Pb, Cu, or Ni (at 30-70% of EC 50 levels) from shortly after fertilization to 72-96 h of development. Under control conditions, whole body Ca concentrations increased approximately 20-fold over this period, accompanied by large changes in unidirectional Ca uptake rates from the water, as measured by  $^{45}\text{Ca}$  influx, and large variations in  $\text{Ca}^{2+}$ -ATPase activity. Whole body Mg concentration also increased, whereas Na and K concentrations exhibited a biphasic pattern. Chronic exposure to each of the 4 metals caused pronounced decreases of Ca uptake rate at the blastula, gastrula, and pluteus stages, with the largest inhibitions occurring at the time of gastrulation.  $\text{Ca}^{2+}$ -ATPase activity and whole body Ca concentrations were also greatly reduced. Mg accumulation was moderately disturbed, whereas effects on whole body Na and K were negligible. However acute challenges with much higher levels of each metal had no effect on Ca uptake rates at any time, indicating that the inhibitions of Ca accumulation during chronic exposure did not occur via direct metal competition for uptake sites. These similar effects of the 4 metals on Ca metabolism occurred despite diverse patterns of metal bioaccumulation, ranging from negligible (Zn, Ni) to modest (Cu) to pronounced (Pb). We conclude that non-competitive interference with calcification may be a unifying feature of metal toxicity to developing echinoderm larvae. (ICA, CDA, ILZRO, IZA, NiPERA, Teck Resources, Vale Canada, Xstrata Zinc, NSERC CRD Program, IDRC-IRC program, CNPQ).

**252 Comparative biogeochemical controls on seawater:sediment nickel bioavailability and lifecycle toxicity to sediment-dwelling amphipods and copepods** G. Chandler, University of South Carolina / Department of Environmental Health Sciences, University of South Carolina / Arnold School of Public Health; J. Ferry, University of South Carolina / Dept. of Chemistry and Biochemistry; K. Washburn, University of South Carolina / Dept. of Environmental Health Sciences; L. He, University of South Carolina; E. Stewart, University of South Carolina / Arnold School of Public Health; C.E. Schlegel, NiPERA; E.C. Rogevich-Garman, NiPERA / Ecotoxicologist. Nickel:sediment biogeochemical controls on bioavailability and comparative toxicity to two key estuarine crustaceans were evaluated using partial to full lifecycle bioassays in fine organic-rich estuarine sediments, and, for comparison, in seawater alone. *Leptocheirus plumulosus* is a marine gammarid amphipod that broods 12-25 young to hatching and then releases them at a size equivalent to most adult meiobenthic copepods (~0.7 to 1 mm). These juveniles spend 2-3 weeks in the meiobenthos as free burrowing sediment ingesters before becoming semi-sessile suspension-feeding tube dwellers in the oxic layer of fine muddy sediments in east coast USA estuaries. *Amphiascus tenuiremis* is a lifetime free-burrowing errant meiobenthic copepod that also dwells in the 0-2 cm oxic layer of estuarine sediments as a sediment ingester and diatom/bacterial grazer. It spans a lifecycle size spectrum from 0.05 to 0.7 mm and is representative of sediment copepods that coexist with *L. plumulosus* and other small macrobenthos. Both species experience similar but importantly different routes of sediment and porewater metal exposure influenced by their feeding and lifestyle ecologies. Divalent metal bioavailabilities in marine sediments are strongly influenced by multiple interactive biogeochemical controls of sulfide, manganese:iron (oxyhydroxides, etc.), quantity/qualities of organic carbon (particulate and dissolved), water ionic strength, temperature, redox, sediment porosity, and intensity of sediment bioturbation and ingestion/processing. Toxicity is driven by metal type and bioavailable concentration, exposure duration, faunal feeding modes, and, for crustaceans, the frequency of molting and amount of body surface area exposed to metal uptake. This study used redox-controlled flow-through sediment microcosms to measure nickel bioavailability and toxicity to *L. plumulosus* and *A. tenuiremis* in the context of each's life history/stage and sediment use. In sediment-free partial lifecycle exposures, juvenile *A. tenuiremis* are 2-3 times more sensitive to nickel than juvenile *L. plumulosus* even though similar in body size. As body size increases with *L. plumulosus* growth, the disparity in X-species nickel sensitivity increases in seawater alone, but for sediment exposures, nickel differential toxicity was attenuated by multiple interactions of biogeochemistry that were measured, predictive, acting at the population level, and interesting.

**253 Is energy metabolism impairment the unifying response to copper exposure in marine invertebrates?** M.M. Lauer, V.C. Azevedo, A. Bianchini, Universidade Federal do Rio Grande – FURG / Instituto de Ciências Biológicas. In freshwater invertebrates and fish, acute copper (Cu) toxicity is associated with ionoregulatory disturbances. We have demonstrated that Cu is also an ionoregulatory toxicant in some euryhaline invertebrates acclimated to low water salinities. However, we have failed to demonstrate any ionoregulatory impairment in euryhaline invertebrates (molluscs and crustaceans) exposed to Cu in sea water. In contrast, several biochemical and physiological effects (changes in oxygen consumption, oxidative stress, and energy metabolism), have been reported in marine invertebrates after acute exposure to Cu. However, most of these effects have been demonstrated using high Cu concentrations. At environmentally relevant Cu concentrations, a unifying mechanism explaining the mode of the metal toxic action in marine invertebrates is still under debate. After observing that the Cu-induced ionoregulatory disturbance observed in copepods acclimated to dilute sea water was ameliorated by feeding, experiments to evaluate the effect of environmentally relevant Cu concentrations on energy metabolism were performed in different groups of marine invertebrates (sea urchins, sea anemones, sea cucumbers, and crabs). Endpoints analyzed included those associated with the activity of key metabolic enzymes (those involved in glycolysis, Krebs' cycle, and the mitochondrial electron transport chain) and oxidative-stress related parameters in respiratory "organs". Changes in membrane potential of mitochondria isolated from crab gills were also considered. Results showed that Cu exposure inhibited the activity of several key metabolic enzymes in all marine invertebrates studied, and disrupted the membrane potential of mitochondria isolated from crab gills. In addition, reduced antioxidant capacity and increased oxidative damage (lipid and protein oxidation) were observed in the respiratory "organs" of the marine invertebrates. These findings clearly indicate that Cu is impairing energy production, with consequent oxidative damage to the respiratory "organs" of marine invertebrates. In light of the above, we suggest that the observed Cu-induced impairment in energy metabolism at the respiratory "organ" could be considered as a unifying mode of toxic action for Cu in marine invertebrates. Implications of the findings reported here for the development of a marine version of the Biotic Ligand Model will be also discussed. [Support: IDRC (Canada), ICA (USA), CNPq and CAPES (Brazil)].

**254 Copper effects on physiology of estuarine guppy *Poecilia vivipara*: understanding mechanisms and potential biomarkers** A.A. De souza machado, Federal University of Rio Grande-FURG / Institute of Oceanography; M. Muller Hoff, Federal University of Rio Grande-FURG / Institute of Biology; R.D. Klein, J. Goulart Cardozo, Federal University of Rio Grande-FURG; M. Mussoi Giacomini, Universidade Federal do Rio Grande – FURG / Instituto de Ciências Biológicas; G.L. Pinho, Federal University of Rio Grande-FURG; A. Bianchini, Universidade Federal do Rio Grande – FURG / Instituto de Ciências Biológicas. In freshwater fish, the mechanism of acute waterborne copper toxicity is reported to be related to ionic and osmotic imbalances, following the inhibition of gill  $\text{Na}^+/\text{K}^+$ -ATPase activity. However, the same mode of toxic action is not always evident in estuarine and marine fish. In the euryhaline guppy *Poecilia vivipara*, gill estuary activity was shown to be not affected by copper exposure. Therefore, we have evaluated the response of a large suite of genetic, physiological and biochemical biomarkers to environmentally relevant copper concentrations. Endpoints were analyzed in tissues and whole-body of guppies exposed (96 h) to waterborne copper (5, 9 and 20  $\mu\text{g Cu/L}$ ) in salt water (salinity 24 ppt). Comet assay score and frequencies of nuclear abnormalities, as well as micronucleated cells were analyzed in peripheral erythrocytes, while copper and ions (Na, K, Ca, Mg and Cl) concentrations were determined in fish whole-body. Antioxidant enzymes [superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GST), and glutathione S-transferase (GST)] activity, metallothionein-like proteins (MTLP) concentration, reactive oxygen species (ROS) production, total antioxidant capacity against peroxyl radicals (ACAP), and lipid peroxidation (LPO) were evaluated in liver, gills and muscle. Results show that guppies were able to regulate the whole-body copper concentration at low copper concentrations, but metal was shown to be accumulated after exposure to 20  $\mu\text{g Cu.L}^{-1}$ . Whole-body Na, K, and Mg disturbances were observed after exposure to copper, suggesting that ionoregulatory mechanisms taking place at gills and likely also kidney were impacted by copper exposure. Also, disturbances of ionic regulation were significantly correlated with the level of oxidative damage in gills ( $r^2=0.97$ ), suggesting an indirect (oxidative) effect of copper on the ion-transporting



mechanisms. Copper exposure also induced oxidative damages to DNA (strand breaks, micronuclei formation and other nuclear abnormalities). CAT activity in liver, ROS, ACAP and LPO in muscle, gills and liver, micronuclei frequency in erythrocytes, as well as whole-body ion (Na, K, and Mg) composition showed to be the most promising biomarkers to assess the exposure of *P. vivipara* to environmentally relevant copper concentrations, since they showed a concentration-dependent response to copper exposure and biological relevance.

**255 Design and Calibration of an AQUATOX Ecosystem Model for Assessing Effects of the Herbicide Prosulfocarb on Aquatic Food Webs** D.A. Rudnick, Integral Consulting, Inc; R.A. Pastorok, Integral Consulting; D.V. Preziosi, Integral Consulting Inc; Y. Bramley, Syngenta; E. Farrelly, Syngenta / Ecological Sciences; D. Huggett, Syngenta; P. Thorbek, Syngenta Ltd / Environmental Safety, Syngenta / Environmental Safety. The use of toxicity experiments in laboratory, mesocosm, and field applications plays an important role in pesticide risk assessment but cannot address all issues related to indirect effects mediated through species interactions within food webs. For example, as part of the European Union registration process for the herbicide prosulfocarb, available laboratory toxicity data and aquatic mesocosm results indicated the potential for short-term effects on phytoplankton and periphyton species, but the nature and time-frame of potential indirect effects on aquatic animals, including fish, were unknown. Aquatic ecosystem modeling can be used to evaluate not only direct toxic effects of pesticides but also indirect effects mediated through trophic interactions of species (e.g. competition and predation). We have developed an aquatic ecosystem model using AQUATOX (updated v. 3.0) to evaluate the potential direct and indirect ecological effects of prosulfocarb on freshwater systems. We report here on the design and calibration of AQUATOX to represent mesocosms originally intended for assessing community-level effects of prosulfocarb. Physical characteristics, nutrient loading, and major biotic groups in the AQUATOX mesocosm-mimic model corresponded to those of the prosulfocarb-study mesocosms. Biological communities included phytoplankton, periphyton, zooplankton, benthic and epiphytic macroinvertebrates, and macrophytes, but not fish as the latter were absent from the mesocosms. To match characteristics of the mesocosms, our mesocosm-mimic model assumes spatial homogeneity in the water column and lacks immigration and emigration. The model was parameterized using data from the prosulfocarb-study mesocosms, from the AQUATOX internal library, and from the open literature. Information on toxicity and environmental fate of prosulfocarb was obtained mainly from the Draft Assessment Report developed by the responsible Rapporteur Member State. Overall, the model is well calibrated (generally within a factor of 2-3) to the mesocosm data, particularly for prosulfocarb concentration and biological variables. We conclude that aquatic ecosystem modeling complements empirical approaches to pesticide risk assessment by allowing efficient evaluation of indirect effects, testing of multiple exposure scenarios, and assessment of potential long-term effects and recovery times.

**256 Accounting for Intended Use Application in Characterizing Contributions of D5 to Aquatic Loadings Following Marketed Personal Care Product Use** B. Montemayor, Canadian Cosmetic, Toiletry and Fragrance Association, Canadian Cosmetic, Toiletry and Fragrance Association / Toiletry and Fragrance Association; B. Price, Procter & Gamble; R. van Egmond, Unilever Research Laboratory Colworth / SEAC. Decamethylcyclopentasiloxane, commonly known as D5 (cyclopentasiloxane) has a wide application of use across a multitude of personal care product categories. The relative volatility of D5 is one of the key properties attributed to this substance that provide for the derived performance benefits attributed to the use of this raw material in personal care formulations. On this basis, rapid evaporative loss following use of many products comprising D5 is expected following typical use application and corresponding wear time. Studies were conducted on three key product categories containing D5 (antiperspirants, skin care products and hair care products) to investigate the amount of D5 that may be destined to go down the drain following simulated typical personal care use scenarios. Marketed antiperspirants and skin care products were applied to human subjects and hair care products were applied to human hair tresses and subsequently rinsed off at designated time points. Wash water was collected at 0, 8 and 24 hours (antiperspirant and hair care analysis) and additionally at 4 hours (skin care analysis) post product application and samples analyzed by isotope dilution headspace gas chromatography/mass spectrometry (GC/MS) to quantify the concentration of D5

destined to be available to go down the drain in captured wash water. It is demonstrated that only a very small fraction of the D5 in products (less than 1%, 0.3 % and 0.02% for antiperspirants, skin care and hair care products, respectively) is available to go down the drain from 'leave-on' type personal care products following typical conditions of use.

**257 The Role of Hazard Property and Exposure Based Approaches in the Assessment of Industrial Substances** M.A. Bonnell, Environment Canada / New Substances Division, Environment Canada / Science and Risk Assessment Directorate. The domestic inventories of industrial chemicals in many countries number in the tens of thousands. Given the global demand to ensure public and environmental safety from commercial chemical use, regulatory agencies have shifted their approaches from lengthy single-chemical assessments, that require substantial resources and time to complete, to rapid evaluation approaches that prioritize the most potentially harmful chemicals for further assessment. Because there are different approaches that can be used to prioritize chemicals, we will examine hazard property driven approaches (e.g., vPvB, PBT) and exposure driven approaches (e.g., risk quotients) and ask the question of when these approaches are most effective when making regulatory decisions in light of the protective goals of the assessments. The decision-making process is very much connected to science/policy driven metrics such as weight of evidence and precaution as well as uncertainty. We will therefore examine the interrelationship of these factors as well. Canada's experience and insights over the last ten years from Categorization, the new chemicals program and other activities under the Chemicals Management Plan will serve as a template for discussion.

**258 Use of Fugacity Ratio Methodology for Assessment of Bioaccumulation Potential: Concepts, Applications, and Caveats** K.B. Woodburn, Dow Corning Corporation / Health & Environmental Sciences; D.E. Powell, Dow Corning Corporation / Health & Environmental Sciences, Dow Corning Corporation / Health & Environmental Sciences (C03101). A historic but infrequently used approach for comparing bioaccumulation (B) data is to express the B assessment metrics in terms of the thermodynamic status of the chemical with respect to a reference phase using fugacity ratios. Participants in a 2009 SETAC Pellston Conference concluded that the fugacity ratio approach is a practical framework for decision making in chemical management and regulation, particularly with regard to assessing bioaccumulation potential. Fugacity is an equilibrium criterion and can be used to assess the relative chemical activities or chemical potentials of a chemical in a specific system. At thermodynamic equilibrium, the chemical fugacities in the different phases are equal. A fugacity ratio between an organism and a reference phase (e.g., water, sediment, or diet) greater than 1 indicates that the chemical in the organism is at a higher fugacity (or chemical activity) than the reference phase. The application of the fugacity ratio methodology has a number of advantages. First, the technique can make use of all laboratory data for which bioaccumulation metrics exist. Secondly, the fugacity ratios are able to express the alphabet soup of bioaccumulation measures (i.e., BCF, BMF, BSAF, TMF) on a similar basis, i.e., as a dimensionless ratio. Third, the fugacity ratios can be visualized in a weight-of-evidence approach, which allows all available data to be viewed together and which also increases confidence in the outcome of the assessment. Most importantly, the fugacity ratio approach provides a formal method for testing the hypothesis whether biomagnification may occur by testing whether fugacity ratios are generally greater than unity (1.0). This presentation will review the fugacity ratio concept, its practical application with a variety of chemical families, how use of fugacity ratios may inform ecological risk assessments, and a discussion of appropriate caveats when applying this technique.

**259 Advances in mechanistic effect modeling can contribute to value-relevant ecological risk assessments** V.E. Forbes, University of Nebraska Lincoln / School of Biological Sciences; P. Calow, University of Nebraska Lincoln / Office of Research and Economic Development. Current methods for ecological risk assessment are often protective, but they are not consistently so. The most common outputs of the risk assessments (i.e., hazard- or risk quotients) are far removed from environmental protection goals and therefore are not as helpful for informing management decisions as they could be. Recent advances in mechanistic effect modeling (which include toxicokinetic/toxicodynamic models, dynamic energy budget models, as well as various types of population models) have demonstrated that spatial and temporal variability in exposure, avoidance and dispersal behavior of

exposed organisms, and differences in life history among species can measurably influence impacts of chemicals on populations and should be more systematically explored in a risk assessment context. An important advantage of such models is that their outputs are more directly related to protection goals, i.e., the outputs are value relevant. In addition, coupling information on the impacts of chemicals on populations or groups of populations to the delivery of ecosystem services, through the development of robust ecological production functions, can provide a scientific basis for more transparent risk management decisions. A key question is whether future risk assessments should add non-traditional information on top of traditional approaches or whether the time is right for a paradigm shift in our overall approach to ecological risk assessment.

**260 The Effect of Pre-exposure on *in Vitro* Biotransformation Rates of Hydrophobic Chemicals in Rainbow Trout (*Oncorhynchus Mykiss*)** L.J. Saunders, Simon Fraser University / Department of Biological Sciences; S.V. Otton, Simon Fraser University / School of Resource and Environmental Management; F.A. Gobas, Simon Fraser University / School of Resource and Environmental Management (Faculty of Environment), Simon Fraser University / School of Resource & Environmental Management, Faculty of Environment, Simon Fraser University. Knowledge of chemical biotransformation rates in fish and other organisms has been identified as a key requirement for assessing the bioaccumulative potential of highly hydrophobic chemicals. There is much effort to develop *in vitro* to *in vivo* extrapolation methods to obtain metabolic transformation rates but a criticism of these methods is that the laboratory animals used as the source of liver metabolizing enzymes for *in vitro* testing may not represent organisms in real environments. The activity of biotransformation enzymes in organisms exposed to multiple pollutants can be inhibited or induced. The present study examined the effect of pre-exposure of rainbow trout (*Oncorhynchus mykiss*) to secondary treated municipal wastewater effluent on the *in vitro* biotransformation rates of polycyclic aromatic hydrocarbons (PAHs). Fish were exposed under static conditions for 7 days to environmentally relevant dilutions of effluent at 0, 0.1, 1 and 10%. PAH biotransformation rates in liver S9 fractions were measured in each exposure group via the conventional substrate depletion method. Pyrene and benzo[a]pyrene depletion rate constants were significantly higher in liver fractions from fish exposed to 10% diluted effluent compared to controls, suggesting that pre-exposure induced the activity of PAH-metabolizing enzymes. The results indicate that *in vitro* tests using laboratory animals may underestimate biotransformation rates occurring in organisms in the field. Using organisms that represent *in situ* situations may provide more accurate laboratory assessment when measuring biotransformation rates in animals for bioaccumulation assessment.

**261 The Chemical Footprint as a Ruler for Impactful Risk Management of Toxic Pollution** M.C. Zijp, National Institute for Public Health and the Environment; D. van de Meent, Radboud University Nijmegen / Institute of Wetland and Water Research; L. Posthuma, RIVM / Lab. for Ecological Risk Assessment, RIVM / LER; A. Hollander, Radboud University Nijmegen / Institute of Wetland and Water Research. The term 'chemical footprint' was coined to express whether gross chemical emissions (separate compounds or mixtures) are larger than the capacity of the environment to accommodate emissions. The concept encompasses a strong communicative element, allowing for evaluating effects of chemical risk management. However, a generally accepted method for deriving a chemical footprint does not seem to exist yet. We have tested the utility of- and expanded the calculation procedures published earlier by Harbers et al (2006) and Eurostat (2009) for this purpose. Earlier calculations have suffered from limited data availability. This technical problem has been solved by REACH, the European Community Regulation on chemicals, implying that substances can be marketed only when safe use has been proven. Substance information is becoming available now from REACH registration dossiers, which have currently been submitted for over 6000 different chemical substances. This information gave us the opportunity to derive the Risk Characterization Ratios (RCReco) for 350 substances representing 60% of the used tonnages in EU. The summed RCReco remained below unity, indicating that all chemicals together exerted a toxic pressure that, produced from one chemical only, would have been considered acceptably low. In addition to this, we have reworked the calculation result into a chemical footprint of EU-wide use of these substances, applying the principles of Species Sensitivity Distributions. Applying the generally accepted criterion that no more than 5% of the species should be exposed to concentrations greater than their NOEC, We

calculated the chemical footprint of the EU to be approximately 50% of the total fresh water capacity in Europe. In other words: half of the existing fresh water ecosystem is occupied to its set maximum capacity. To summarize we used the following steps to derive the chemical footprint for Europe: 1) hazard definition: scale and endpoint (e.g. biodiversity in fresh waters); 2) sub-selection of chemicals; 3) exposure and fate modeling; 4) dose effect modeling; 5) derive the multi substance Potentially Affected Fraction of species; 6) conversion of this to the footprint unit. During the presentation, the relative and absolute meaning of the outcomes of the classical RCR and the footprint method are discussed and usages of chemical footprints on product-, organization- and regional level will be evaluated.

**262 Standard and non-standard ecotoxicity data in risk assessment of chemicals** M. Agerstrand, Royal Institute of Technology (KTH) / Div. of Philosophy; M. Breitholtz, ITM, Stockholm University; C. Ruden, Royal Institute of Technology (KTH). Guidance documents for pharmaceuticals, plant protection products, biocides and industrial chemicals recommend the use of standard ecotoxicity tests in regulatory risk assessments. These recommendations have decreased the use of non-standard test data. On the positive side, this has increased the reliability of the data since the strict reporting requirement for standard tests enhance reliability, and thereby the reproducibility of the data. On the negative side, it could have implications for the relevance of the risk assessment. Standard tests may not always be sensitive enough to measure the specific effects expected for instance from endocrine disrupting chemicals, nanoparticles and pharmaceuticals. Bisphenol A is an illustrative example since three standard tests are preferred by several risk assessors over 200 non-standard studies with lower effect values. Previous studies indicate that ecotoxicity studies published in the open scientific literature are surprisingly often insufficiently reported. This may be a major reason why they are often seen as less reliable in a risk assessment context. Choice of reliability evaluation method could also affect the outcome of the evaluation. Several guidance documents recommend that the method described by Klimisch et al. (1997) should be used for evaluating the reliability of ecotoxicity and toxicity data but the Klimisch criteria give a strong preference for standard tests. We present a novel and more comprehensive method for evaluating and reporting non-standard ecotoxicity data. Its aim is to enable an increased use of non-standard data in risk assessments. As part of this work we clarify the definitions of "reliability" and "relevance" in order to promote the consistent use and application of these concepts in risk assessment procedures.

**263 Purpose of the Special Symposium "21st Century Environmental Risk Assessment"** J. Toll, Windward Environmental LLC; M.S. Johnson, US Army, Public Health Command / Health Effects Research Program, US Army Institute of Public Health / Health Effects Research Program, Army Institute of Public Health / Health Effects Research Program, US Army Ctr for Health Promotion & Preventive Med. / Health Effects Research Program. The special symposium "21st Century Environmental Risk Assessment" is meant to help kick off a new Ecological Risk Assessment Advisory Group (ERA EG) work group on solution-focused risk assessment. The idea of forming the work group was inspired by the U.S. National Research Council's Silver Book (*Science and Decisions: Advancing Risk Assessment*, National Academies Press 2009), particularly Chapter 8, "Improving the Utility of Risk Assessment." A brief article about this appeared in the February 2012 *Globe* ([www.setac.org/globe/2012/february/era.html](http://www.setac.org/globe/2012/february/era.html)). SETAC's ERA AG should play a role in vetting ideas about improving the utility of environmental risk assessment, identifying good ideas that we think should be implemented, and promoting those ideas. That includes ideas about how to improve the environmental risk assessment framework and ideas about better risk assessment tools. That's what this special symposium and the work group on solution-focused risk assessment are about.

**264 Solution-Focused Risk Assessment and Its Challenges** B.K. Hope, CH2M Hill. Solution-focused risk assessment (SFRA) is an attractive idea, and one that may play a role in environmental risk assessment, provided it can overcome certain challenges. My insights into these challenges stem from being a member of the National Research Council committee that considered SFRA and from having reviewed and critiqued the first extended explication of it. SFRA's fundamental premise is that, after sensing a problem (i.e., a "signal of harm"), you first consider possible solutions, then examine risks and benefits associated with each potential solution. This is in opposition to the current paradigm that senses a signal then uses risk

assessment to analyze and characterize it (and perhaps turn a non-problem into an illusory big one). SFRA's first challenge lies with how that "signal of harm" is sensed, by whom, and whether there's agreement as to its magnitude or very existence. A second challenge, should that signal's existence and magnitude be agreed, is discerning its source. This may be a simple task or a legally complex matter of attribution and responsibility, but is an essential one if resources are to be found to consider and implement solutions. A third challenge, which applies to both problem- and solution-focused approaches, is the assessment itself. If it embodies overly conservative assumptions, it may turn a small signal into a large one and thus distort the risk/benefit ratio for different solutions. And, if it is a dichotomous hazard, rather than a true, risk assessment, it will not address the continuous relationships between exposure and consequence envisioned by SFRA. These challenges notwithstanding, a realistic path to SLRA may be possible if we used the extant first (albeit often *pro forma*) step in risk assessment – problem formulation – to meet SLRA's requirement that risk managers and assessors take time to consider solutions before analysis and opportunities before problems.

**265 Integrating adaptive management into the risk assessment process** L. Barnthouse, LWB Environmental Services, Inc.. The National Academy of Sciences' 2009 report *Science and Decisions* made an important contribution to risk assessment by providing a framework for ensuring that risk assessments support management decision-making. The 2009 report expands on recommendations made in a 2005 Academy report on risk assessment and risk management at large Superfund sites. This earlier report similarly stressed the need for risk assessment to support management decision-making. A key recommendation of the 2005 report was that EPA adopt adaptive management as a strategy for restoring sites that cannot be managed using the conventional RI/FS process because the size and complexity of the sites makes it impossible to design a "final remedy" prior to the initiation of remedial actions. At such sites, a phased approach to remediation, often accompanied by research on site characteristics or treatment technologies, is necessary to achieve success. The concept of adaptive management, originally developed more than 40 years ago and now widely utilized by resource management agencies, provides a systematic means of designing and implementing environmental management programs for these types of sites. The term "adaptive management" generally describes an approach to environmental resource management in which information obtained during the implementation of a management action is used to refine the action and ensure that it meets management objectives. Key technical elements of adaptive management include conceptual or quantitative models that are used to predict the outcomes of actions, performance metrics and targets for evaluating the success or failure of actions, and contingency plans for responding to unexpected outcomes. Key institutional elements of adaptive management include institutional commitment and active involvement of stakeholders. To be effective, however, adaptive management must be applied throughout all phases of the risk assessment and risk management processes, from problem formulation through risk characterization and beyond. Using examples from actual sites, this paper identifies requirements for successfully applying adaptive management principles to complex risk assessment and risk management activities.

**266 Science and Decisions in the EPA's Risk Assessment Forum** G.W. Suter, U.S. Environmental Protection Agency / National Center for Environmental Assessment, U.S. EPA / National Center for Environmental A; L. Martin, U.S. EPA / Risk Assessment Forum. The National Research Council's (NRC) report *Science and Decisions: Advancing Risk Assessment* was commissioned by the U.S. Environmental Protection Agency as an update to the 1983 red book. Since its publication, the Agency's Risk Assessment Forum (RAF) and National Center for Environmental Assessment have taken the lead in implementing its recommendations. Most notably, the NRC replaced the old framework for human health risk assessment with a more complex one that is concerned with decision support and adopts the concept of problem formulation from the ecological risk assessment (ERA) framework. The RAF is developing a human health risk assessment framework that is responsive to the NRC. On the ecological side, the RAF organized a colloquium on ecological risk assessment that developed recommendations for advancing ERA and subsequently condensed that into an Ecological Assessment Action Plan. The plan includes major, policy-relevant projects on using ecosystem services as assessment endpoints, communicating ecological risks, inference by weight of evidence, integration of ecological

assessments, applying adaptive management to environmental protection, and strengthening ecological goals. Proposed projects related to technical practices include improved training and access to information, quality assurance, multiple stressors, and uncertainty characterization and communication. There are also collaborations between human health and ecological assessors on cumulative risk, uncertainty, and terminology. SETAC members are providing input to these projects and they should find the results to be useful in various contexts. *The views expressed in this abstract are those of the authors and do not necessarily represent the views or policies of the U.S. Environmental Protection Agency.*

**267 Building Opportunities for Habitat Restoration or Control of Invasive Species into Environmental Risk Assessments** K.G. Keil, US Army Corps of Engineers / Buffalo District, US Army Corps of Engineers; L.C. Carroll, US Army Corps of Engineers. The U.S. Army Corps of Engineers performs environmental risk assessments for at least two types of projects: hazardous, toxic, or radioactive waste (HTRW) sites, as well as non-HTRW sites (dredged material disposal). The HTRW sites may be part of a Base Realignment and Closure site, a Defense Environmental Restoration Program site, or a Formerly Utilized Sites Remedial Action Program site (sites with residual radioactivity from the Manhattan Project). The non-HTRW project sites result from maintenance dredging of U.S. waterways, and may involve engineered confined disposal or unconfined upland placement. In following our mission to ensure that our project sites are fully protective of human health and the environment, the EPA's Environmental Risk Assessment (ERA) framework is used to determine whether or not contamination at a site may pose an unacceptable risk to ecological receptors. However, many of these sites are undergoing redevelopment as land uses change, and the traditional EPA ERA framework often does not serve overall project objectives to strive for green remediation, sustainable development, or ecosystem restoration. Sites with little to no habitat are allowed to "by-pass" the ERA process altogether (reasoning that there are not any complete ecological exposures pathways), even though these sites could provide opportunities for habitat creation. Furthermore, sites with disturbed but recovering habitat may "fail" the ERA (due to presence of low level contamination) but can provide great opportunities for ecosystem restoration or enhancement through invasive species control projects. Thus the ERA is often performed but then set aside as decisions are made for the project to either remediate the site to protect human health (in the case of many HTRW brownfield sites) or continue with ecosystem improvements (only using the ERA indirectly). The USACE's Environmental Operating Principles challenge us to "strive to achieve environmental sustainability" and to "seek balance and synergy among human development activities and natural systems". Those guidelines should be reflected somehow in the ERA process. Several case studies of HTRW and non-HTRW sites will be presented and the associated ERA process will be evaluated to determine what adjustments in the ERA would be useful in providing information that would assist in actually improving environmental conditions at the project site.

**268 Looking Back to See Ahead: The Current and Future Role of Ecological Risk Assessment in Sediment Remedial Decisions** K.B. Bradley, GEI Consultants, Inc. / Ecologist, GEI Consultants / Project Scientist/ Ecologist; B.W. Gensemer, GEI Consultants / Ecological Division; B. Hermanson, GEI Consultants, Inc.; S.A. Roark, GEI Consultants. Incorporation of developing risk assessment methodologies and models into solution-based ecological risk assessments (ERAs) can only be successful if these approaches are integrated into the remedial investigation/feasibility study (RI/FS) process. Sediment remediation projects often include ERA approaches based upon recent scientific advances, only to have remedial decisions, even preliminary remediation goals (PRGs), selected for practical feasibility, cost, or risk-based conservatism, rather than relying on information from the ERA. This is primarily due to the use of the pre-1988 paradigm for the RI/FS process, where the RI and FS work in series. The RI/ERA step focuses on characterizing the site and quantifying risk, with little regard for potential remedies. The focus on characterization and risk identification relies primarily on a goal of scientific integrity in the analysis, and production of a high degree of certainty about the characteristics of the site. The FS step then introduces practical issues, defining risk management options based on the problems defined in the previous step with concerns about feasibility and cost efficiency. In this presentation several high profile the sediment remediation projects will be reviewed to highlight the use of the old RI/FS paradigm, and identify the points of disconnect between the



science-based conclusions resulting from the ERA and the ultimate remedial decisions made from the RI/FS process. The presentation will then point to the “new” parallel process paradigm of the RIFS process established in 1988, and show how solution-based ERA guidelines that focus on an integrated risk-based decision making process will provide better information throughout the RI/FS process.

**269 Some Practical Ideas for Accelerating Progress at Contaminated Sediment Sites** J. Toll, Windward Environmental LLC. The “top five actions” presented by Todd Bridges, Steve Nadeau and Megan McCulloch in “Accelerating Progress at Contaminated Sediment Sites: Moving from Guidance to Practice” (IEAM 8(2):331-338) represent an important set of normative guidelines that could accelerate progress at contaminated sediment sites. An important next step is to use those guidelines to develop recommendations about good practices. This presentation will offer some recommendations of that sort, paired with the guidelines from Bridges et al. that inspired them. The objective is to draw more attention to solving the important problem of how to accelerate progress toward cost-effective cleanup, closure and beneficial future use of contaminated sediment sites.

**271 Computational Toxicology: A More Efficient and Effective Testing and Assessment Paradigm for Chemical Risk Management** S.P. Bradbury, U.S. Environmental Protection Agency / Office of Pesticide Programs Office of Chemical Safety and Pollution Prevention. A major challenge confronting chemical risk management is to address the potential ecological and human health risks for large numbers of chemicals with greater speed and accuracy using fewer resources. From a strategic and tactical viewpoint, an integrated approach that relies on existing knowledge (including mechanistic information of both chemistry and biology from novel computational toxicology) is needed to advance progressive and focused assessment strategies, as well as to advance the utility of risk assessment by providing more relevant information. This presentation will describe the use of “Integrative Approaches to Testing and Assessment” is a means to efficiently use existing exposure and toxicity information combined with (Q)SAR predictions and in vitro data in a systematic and coordinated manner for a chemical or a group of similar compounds.

**272 Using Transcriptomic Data in the Risk Assessment Paradigm** R.S. Thomas, The Hamner Institutes for Health Sciences / Institute for Chemical Safety Sciences, Center for Genomic Biology and Bioinformatics. The integration of transcriptomic data into the risk assessment paradigm is a challenge currently facing risk assessment. To assess the value of applying transcriptomics in risk assessment, a series of rodent studies were performed across five chemicals that were positive for lung or liver tumors in a standard cancer bioassay, with transcriptional changes in the lung or the liver evaluated using microarrays. The dose-response changes in gene expression were analyzed using standard benchmark dose methods that have been commonly employed in risk assessment. A comparison of the transcriptional benchmark dose values with those for the traditional noncancer and cancer apical endpoints was conducted showed a high degree of correlation for specific signaling pathways. Many of the correlated pathways have been implicated in noncancer and cancer disease pathogenesis. The results demonstrate that transcriptomic changes in signaling pathways can be used to estimate noncancer and cancer points-of-departure for use in quantitative risk assessments.

**273 Adverse outcome pathway Adverse Outcome Pathways as a Unifying Concept in Environmental Toxicology** K.M. Crofton, ORD, US EPA / Integrated Systems Toxicology Division, NHEERL. An adverse outcome pathway (AOP) is a subset of a source to outcome pathway that delineates documented and testable linkages between a molecular initiating event and an adverse outcome at the individual or population levels. AOPs, by definition, span multiple levels of biological organization. The amount of detail and linearity characterizing the AOP between a molecular initiating event and an adverse outcome within an AOP can vary substantially, both as a function of existing knowledge and risk assessment needs. Development of AOPs can provide insight into the uncertainties in linking chemical use, exposure and outcome, thereby focusing research on critical data needs. Well defined AOPs provide utilitarian framework to build quantitative and qualitative models useful in: understanding the degree of perturbation associated with adverse outcomes, target for the development and use of efficient chemical testing methods, and extrapolation between species and lifestages.

The development and use of AOPs can provide a framework in which high-throughput and high-content information informs more efficient and predictive risk decisions.

**274 Will Global Climate Change Influence the Conduct of Human and Ecological Risk Assessments for Contaminated Sites?** R.G. Stahl, DuPont Company / Corporate Remediation Group; T. Bingman, A. Guiseppe-Elie, DuPont Company / Corporate Remediation Group. The potential for global climate change (GCC) to impact humans and ecosystems has been discussed in multiple scientific and policy forums in past years, and in numerous published articles in the scientific and lay press. In 2011 SETAC sponsored an international, Pellston workshop on the potential for GCC to influence the scientific underpinnings of environmental toxicology and chemistry, and results from that effort have been presented in 2011 at SETAC North America (Boston), and in 2012 at SETAC World Congress (Berlin). Results from these efforts indicate that GCC is likely to manifest as a series or suite of stressors that may require changes in human and ecological exposure scenarios, or in estimating the magnitude of chemical impacts on humans and ecosystems. With this in mind, we have considered the question of whether or not GCC will influence the conduct of human and ecological risk assessments for contaminated sites. In this presentation we will discuss the potential for GCC to require changes in human and ecological exposure scenarios for contaminated site assessment, as well as the potential to influence risk management decisions to protect humans and ecological receptors.

**275 Integrating Emerging Technologies into Chemical Safety Assessment** L. Ritter, University of Guelph / Canadian Network of Toxicology Centres. In response to a request from the Pest Management Regulatory Agency of Health Canada, the Council of Canadian Academies convened an expert panel to examine the use of integrated approaches to testing and assessment (IATA) for the regulatory risk assessment of pesticides. The Panel concluded that, although there is no set of alternative tests that can completely replace the existing battery for data-rich chemicals today, the state of the science is evolving rapidly. IATA offers a transparent and scientifically robust framework to integrate new scientific knowledge into regulatory toxicity testing in a way that significantly augments the protection of human health and the environment. This, in turn, will help address the risk communications challenge, which will be instrumental in establishing public confidence and trust. The Panel concluded that transparency, coupled with sincere and early engagement of all stakeholder groups, will be instrumental in establishing public confidence and trust. The Panel also concluded that advances in computational sciences have established a foundation that may permit the use of epidemiological data in pre-market assessment of structurally related chemicals. This, coupled with advances in biomarker development, represents an exciting opportunity to address a major criticism in the existing approach. The report offers a near-term perspective, focusing on strategies that could be adopted over the next decade. Ongoing implementation would help move toxicology away from asking *what* and towards explaining *how*. The state of science is evolving rapidly and opportunities exist to address some of the limitations that were not, until now, addressable. Furthermore, continued advances will likely identify new limitations that were previously unidentified. These 21st century problems will need 21st century solutions. Although IATA may not be able to address all of these issues, it represents a transparent and pragmatic blueprint by which change may be effected.

**276 MABEL: use of preclinical data to set acceptable standards for exposure** G. Bruce, Intertox. The growing availability of sensitive pharmacological data and preclinical data characterizing the agents' mechanisms of action presents a unique opportunity to explore their use in developing screening levels for these compounds. This presentation will demonstrate use of the Minimum Anticipated Biological Effect Level (MABEL)—the lowest dose with any measurable effect on biological systems—an alternative to deriving a point of departure for ADIs for emerging compounds, particularly PPCPs, in drinking water.

**277 Management of risks from cumulative exposures to chemicals: Moving beyond TEQs and Hazard Indices** P.S. Price, The Dow Chemical Company / Toxicology & Environmental Research & Consulting. The human health and ecological risks posed by cumulative exposures to chemicals, including exposure to mixtures, have traditionally been investigated using

additive models. These models include screening models such as the Hazard Indices, systems of TEQs that are based on a common mechanism of action, and Toxicity Units that are based on empirical measurements of acute toxicity endpoints. More recently the IPCS/WHO has proposed a tiered framework of modeling approaches for the evaluation of mixtures. This framework proposes the use of both screening assessments and higher tiered approaches that consider detailed mechanisms of joint action and or use of probabilistic models of toxicity and exposure. The European chemical industry (Cefic) has developed a Decision Tree approach that extends the IPCS/WHO framework to include a determination of the value in performing cumulative assessments. These new tools provide increased flexibility in the evaluation of risks from mixtures. They allow the use of screening evaluations that can be performed with minimal data and describe how additional data can be used to refine the screening assessments. Finally, the new tools allow the identification of specific groups of chemicals and specific populations where cumulative risk assessments are most needed.

#### **278 The Case for Activity and Fugacity Based Environmental Risk Assessment: The Example of the Plasticizer DEHP** *EA Gobas*, Simon Fraser University / School of Resource and Environmental Management (Faculty of Environment), Simon Fraser University / School of Resource & Environmental Management, Faculty of Environment, Simon Fraser University; V. Otton, Simon Fraser University; K. Clark, BEC Technologies Inc.; M. Ikononou, Institute for Ocean Sciences. A novel application of thermodynamic activity and fugacity in risk assessment is presented to assess the environmental risks of the common plasticizer di-ethylhexyl-phthalate ester (DEHP) and its main metabolite mono-ethylhexyl-phthalate ester (MEHP). The theoretical basis of activity based risk assessment is the expression of exposure and toxicity concentrations in similar units of activity and/or fugacity. This approach enhances the data base used in environmental risk assessments by including concentration data from different environmental media and toxicity data from in-vitro and in-vivo studies in one single risk analysis. The approach also identifies erroneous concentrations measurements that cannot exist in the environment. The application of this risk assessment methodology is illustrated for the common plasticizer DEHP and its metabolite MEHP. The risk assessment is based on the compilation of hundreds ( $n=1129$ ) of measured exposure concentrations in air, water, sediment soil, fish and wildlife species and the review of large numbers of toxicity studies by the EU, the US Consumer Product Safety Commission and Staples et al. (2011) in a variety of biological species and in a range of in-vitro systems. The risk analysis is based on the analysis of all available exposure and toxicity data in a single graphical presentation and probabilistic analysis. The risk analysis shows that environmental risks of DEHP and MEHP are very low.

**279 Impacts of Sediment Resuspension on Metal Bioavailability and Toxicity Assessments** *K.J. Farley*, Manhattan College / Civil and Environmental Engineering, Manhattan College / Civil & Environmental Engineering; R. Bubnyte, M.P. Villarosa, Manhattan College / Civil & Environmental Engineering; R.F. Carbonaro, Manhattan College / Civil and Environmental Engineering, Manhattan College / Department of Civil and Environmental Engineering, Mutch Associates, LLC; K.J. Rader, Mutch Associates, LLC, Manhattan College / Civil and Environmental Engineering. The presence of sulfides in sediments has been shown to play an important role in sequestering toxic metals (e.g., Cd, Cu, Ni, Pb, Zn) and in limiting metal bioavailability. This effect has been considered in regulatory decision-making through static or "snap-shot" measures of simultaneously extractable metals (SEM) and acid volatile sulfide (AVS) ratios. A potential weakness in this approach is the oxidation of metal sulfides and associated changes in metal bioavailability that are likely to occur during sediment resuspension events (e.g., due to episodic storms, propeller-induced disturbances and dredging activities). Our colleagues have therefore been examining metal release during resuspension in laboratory chamber studies with sediments from the Anacostia River (Maryland), Lake DePue (Illinois) and San Diego Bay (California). In companion studies, we have used the laboratory results in constructing a one-dimensional model of resuspension events using the Tableau Input Coupled Kinetic Equilibrium Transport (TICKET) model. A large part of this work has been the comparison of laboratory chamber and TICKET modeling results to assess our ability to model metal behavior during resuspension events and to evaluate the relative importance of various physical and chemical processes in controlling metal release. Overall results show that coupled kinetic, equilibrium, transport models such as TICKET

provide an appropriate framework for evaluating the complexities of metal behavior during resuspension events. Results also show that metal release and bioavailability during resuspension is intricately linked to the mass of resuspended sediment, metal sulfide oxidation rates, carbonate buffering, and metal binding to natural organic matter and oxide surfaces. Based on these findings, SEM/AVS, BLM and other regulatory assessment tools will need to be considered in a more dynamic framework to properly address the potential impacts of metals during sediment resuspension events.

**280 Lead bioavailability to soil invertebrates as affected by soil physico-chemical characteristics** *R. Lanno*, Ohio State University / Department of Evolution, Ecology, and Organismal Biology; K. Albanese, The Ohio State University / Environmental Science Graduate Program; J. Chowdhury, International Lead Zinc Research Organization (ILZRO); K. Oorts, ARCHE (Assessing Risks of CHEmicals). The bioavailability and toxicity of lead (Pb) to terrestrial invertebrates may be markedly modified by variations in the physicochemical properties of soil. Other factors that modify the toxicity of Pb include aging and leaching, yet most toxicity data are obtained from toxicity tests conducted with soils freshly amended with metal salts and the relevance of data from such tests to contaminated field soils is unclear. This study compares the chronic toxicity of Pb to *Eisenia fetida* and *Folsomia candida* in six European soils varying widely in physicochemical properties (i.e., pH, organic matter content, texture, eCEC). Toxicity across all six soils was compared using soils that were leached and pH corrected after freshly spiking with lead chloride. This was done to exclude the confounding factors of increased ionic strength and decreased pH due to spiking. Two of these soils (Barcelona, Woburn) were also amended with lead nitrate in 2004 and aged under field conditions for five years prior to conducting laboratory toxicity tests and additionally tested directly after freshly spiking (without leaching or pH correction). These treatments allow for the estimation of leaching/aging factors to extrapolate toxicity from laboratory to field conditions. Significant positive correlations were observed between  $\log(\text{EC}_{50})$  for earthworm reproduction and  $\log(\text{eCEC})$ , total C, exchangeable Ca and Mg, clay) of the soils, but toxicity of Pb to *F. candida* was low and not affected by soil properties. Ageing processes generally decreased the toxicity of Pb to soil invertebrates, but the effects of leaching compared to the toxicity of freshly spiked soils were less distinct. The toxicity data, together with leaching and aging correction factors, provide important information on the bioavailability of lead to invertebrates in soil under realistic conditions.

**281 Modeling the Transport, Effects and Fate of Metal Concentrates in a Lake Using the TICKET Unit World Model** *R.F. Carbonaro*, Manhattan College / Civil and Environmental Engineering, Manhattan College / Department of Civil and Environmental Engineering, Mutch Associates, LLC; K. Delbeke, European Copper Institute; K.J. Rader, Mutch Associates, LLC, Manhattan College / Civil and Environmental Engineering. When evaluating the transport, effects and fate of metals in the aquatic environment, the form of the metal released plays a critical role. Toxicity assessments typically focus on dissolved metal while intermedia transport and ultimate loss of metal from the aquatic system via burial is principally a function of particulate metal. Therefore, one would expect different impacts from metals released as soluble salts (e.g.,  $\text{CuSO}_4$ ), which dissolve instantaneously and particulate metal concentrates/powders/massives, which release metals to the dissolved phase at characteristic kinetic rates. Numerical models can provide insight into the transport, effects and fate of both types of metals and assist in regulatory decision-making. They are particularly useful in grappling with the added complexity associated with kinetic dissolution of concentrates/powders/massives. A unit world model for metals in lakes, TICKET-UWM, has been developed that considers key processes affecting metal transport, fate, and toxicity including complexation by aqueous inorganic and organic ligands (e.g., DOC), adsorption to particulate organic carbon (POC), binding to biological receptors (biotic ligands), and transport of dissolved metals and solids between the water column and sediment. TICKET-UWM was used to model the dynamics of two metal concentrates released in the water column of a generalized lake. Transformation/dissolution data for these two concentrates were used to parameterize kinetic rate expressions for dissolution in the model. Settling rates for the concentrates were estimated using particle size and density data. Simulations were made to examine metal removal from the water column, metal speciation changes upon entering the sediment and the remobilization potential of metals in sediment. Model results indicate that settling of concentrates limits the direct impact dissolution had on water column metal concentrations.

Continued dissolution of concentrates in the sediment did affect the water column indirectly as some of the additional metal released was recycled to the water column through transport processes. Results generally indicate that metal removal from the water column was rapid.

### 282 Predicting Ni toxicity to plants: Comparison of three approaches

Y. Gopalapillai, Vale Canada Limited / School of Environmental Sciences, Natural Resources Canada / CANMET Mining and Mineral Sciences Laboratories, Natural Resources Canada / Metal and Mineral Sciences Laboratories; B. Vigneault, Natural Resources Canada / CANMET, Natural Resources Canada; B. Hale, University of Guelph / School of Environmental Sciences. Models that predict toxicity based on some estimate of the true exposure to a contaminant in media can be cost-effective tools for reliable and rapid risk assessment of metals in soils, water, air and foods. The challenge lies in defining how 'true exposure' is measured or estimated, as for both organic and inorganic contaminants, the characteristics of the exposure medium and the physiology of the organism can profoundly alter what would otherwise likely be a simple relationship between external dose and response. Currently, most Ni risk assessment is based on  $[Ni_{tot}]$ , but, as for many trace elements, it is not considered a good predictor of toxicity since the metal's speciation in the exposure media has the potential to greatly affect its bioavailability to the organism. Three currently available modeling approaches (Biotic Ligand Model (BLM) framework, Predicted No Effect Concentration (PNEC) and Tissue Residue Approach (TRA)) were compared for their ability to predict Ni toxicity to plants, either in aquatic culture (*Lemna minor*) or terrestrial species in soil culture (*Avena sativa*). All three of these approaches are underpinned by the same fundamental mechanism, i.e. that toxicity is related to the 'true exposure'; they differ in their approach to estimating 'true exposure'. Generally, the results indicate that the major assumptions behind the three main modeling approaches do not fully apply to Ni toxicity in the tested plant species (*L. minor* or *A. sativa*). The BLM's assumption of competitive cation interaction does not apply to *L. minor*; PNEC's assumption of the normalizing effect of CEC on toxicity based on total [Ni] does not apply to all soil types; and finally, TRA's assumption that [Ni] in plant tissue is the best predictor of toxicity is not valid for Ni accumulation in *L. minor*, as the more practical total [Ni] in solution better related to observed plant response. Although some water chemistry effects on Ni toxicity to *L. minor* were observed, these effects did not cause large enough changes in the relationship between total [Ni] in solution and Ni toxicity over the wide range of water chemistry tested. Thus, we conclude that total [Ni] is sufficient for rapid and practical assessment of the risks posed by Ni contamination to aquatic and terrestrial plants (sandy and till clay soils).

**283 Update of Water Quality Criteria for Molybdenum** H. Latimer, J. Diamond, Tetra Tech, Inc.; J. Flippin, Tetra Tech, Inc. / Center for Ecological Sciences. This research team developed acute and chronic water quality criteria for the protection of aquatic life for molybdenum for the State of Nevada in 2008, which were subsequently adopted as state standards. More recently, the State of Pennsylvania has proposed adoption of a slightly modified version of these criteria as state standards. In the intervening years two studies have been published in the peer-reviewed literature that provide toxicity data both for species considered in the 2008 criteria and for species for which no data were available in 2008. Using current US Environmental Protection Agency (EPA) criteria development methodology and updating the 2008 criteria to include these new data resulted in a slight increase in the final calculated acute criterion and a significant increase in the chronic criterion. The nature of the resulting changes to these updated criteria are attributable to the generally low toxicity of molybdenum (e.g., the four lowest mean chronic values in the updated database range from 36 to 86 mg/L), the relatively small database (acute data for 14 genera and chronic data for 10 genera), and the limited variability among final toxicity values. This work highlights three elements that should be considered by researchers and regulators when considering new or updated water quality criteria: 1) all water quality criteria considered for adoption as standards should be supported by a recent review of the scientific literature, 2) generation of data for even a few additional genera can have a large impact on the final criteria value for compounds with a fairly limited toxicity database, and 3) the variation among the toxicity values as well as the values themselves play a substantial role in calculating final criteria values using US EPA methodology.

### 284 Development of BLM-based Ambient Water Quality Criteria for Lead following USEPA Guidelines

D.K. DeForest, Windward Environmental LLC; K.A. De Schampelaere, Ghent University; K.V. Brix, McMaster University; B. Church, Windward Environmental LLC; R. Blust, University of Antwerp; M. Chowdhury, International Lead Zinc Research Organization; R.C. Santore, HDR|HydroQual, Inc.. The United States Environmental Protection Agency's (USEPA's) current ambient water quality criteria for lead in fresh water are adjusted for hardness and were last updated in 1985. Since then, there have been two primary advancements in the assessment of lead toxicity to aquatic organisms. First, the acute and chronic toxicity databases for freshwater organisms have expanded substantially, thereby allowing us to explore the chronic toxicity data for lead relative to the USEPA's minimum phylogenetic diversity requirements for deriving criteria. Second, the influence of water chemistry on lead bioavailability has been evaluated in several studies with multiple species, which coincides with the development and validation of acute and chronic biotic ligand models (BLMs) for lead. Using the lead BLMs, acute and chronic species sensitivity distributions (SSDs) based on the geometric mean critical accumulation concentrations for each species and exposure type were constructed and the 5<sup>th</sup> percentiles of these SSDs were derived following the statistical approach described in the USEPA guidelines. Application of these 5<sup>th</sup> percentile acute and chronic critical accumulation concentrations allow for the estimation of the 5<sup>th</sup> percentile dissolved lead concentrations for a broad range of water chemistries (analogous to the USEPA's final acute and chronic values). Unlike other metals for which BLM-based criteria have been developed (e.g., copper, zinc), there are marked differences between organisms in response to water quality parameters. For example, the influence of hardness on lead toxicity is not consistent between species, but dissolved organic carbon (DOC) plays a primary role in protecting aquatic organisms against lead toxicity. A comparative analysis of BLM-based and hardness-based criteria for lead will be provided.

### 285 A Biotic Ligand Model based revision to the U.S. water quality criteria for copper in saltwater for marine and estuarine organisms

R.C. Santore, HDR | HydroQual, Inc.; A.C. Ryan, HDR | HydroQual; R. Arnold, Stephen F. Austin State University; A. Bianchini, Universidade Federal do Rio Grande – FURG / Instituto de Ciências Biológicas; G. Rosen, SPAWAR Systems Center Pacific / Environmental, SPAWAR Systems Center Pacific / Scientist, SPAWAR Systems Center / Scientist; S. Smith, Wilfrid Laurier University / Department of Chemistry, Wilfrid Laurier University / Chemistry; C. Delos, US Environmental Protection Agency, U.S. EPA / Mail Code 4304, US Environmental Protection Agency. A recent update to the water quality criteria for copper in saltwater has been completed. As part of this effort, a literature review was conducted to update the toxicity database used to develop the species sensitivity distribution. Over 700 toxicity endpoints were obtained from this review suitable for inclusion in the marine document. These data represent toxicity information for 80 species in 67 genera, compared to 33 species in 26 genera that were used in the previous document. In the updated database, the most sensitive organisms to copper toxicity were embryo-larval forms of marine invertebrates including *Mytilus edulis*, *Mytilus galloprovincialis*, *Strongylocentrotus purpuratus*, *Dendroaster excentricus*, *Crassostrea gigas*, and *Crassostrea virginica*. There was also considerable evidence in recent literature showing the importance of bioavailability in determining copper toxicity to these sensitive marine invertebrates. Measurements of copper toxicity and copper accumulation in sensitive embryo-larval forms of these marine invertebrates are reduced in the presence of NOM, suggesting that NOM-bound copper is not bioavailable. A marine Biotic Ligand Model (BLM) for copper was calibrated to predict the speciation of copper in marine waters, and accumulation of copper in sensitive invertebrates. The marine BLM for copper was used in this update to incorporate bioavailability effects in the marine criteria, using a methodology that was similar to the most recent update to the freshwater ambient criteria for copper. For the marine invertebrates used to develop and test the marine BLM, little is known about bioavailability effects in estuaries where variation in salinity and pH are common. In this presentation, the marine BLM for copper will be applied to several estuarine species across salinity gradients to evaluate whether the marine BLM is predictive and protective for sensitive estuarine organisms.

**286 Toxicogenomic Screening of Environmental Pollutants on Toxicity** X. Zhang, Toxicology centre, University of Saskatchewan / State Key Laboratory of Pollution Control and Resource Reuse & School of the



Environment, Nanjing University / School of the Environment, State Key Laboratory of Pollution Control and Resource Reuse & School of the Environment, Nanjing University / School of Environment, Nanjing University / School of Environment; G. Su, State Key Laboratory of Pollution Control and Resource Reuse & School of the Environment, Nanjing University; J.P. Giesy, University of Saskatchewan / Biomedical Veterinary Sciences & Toxicology Centre, Michigan State University / Department of Animal Science, University of Saskatchewan / Toxicology Centre; H. Yu, State Key Laboratory of Pollution Control and Resource Reuse & School of the Environment, Nanjing University. The number of new chemicals or materials developed in industry keeps growing rapidly every year and their chemical structures are heterogeneous. There is urgent need for development of high throughput methods that allow evaluation of large numbers chemicals that can enter the environment for their potential toxicity and to prioritize these chemicals for further toxicity testing. However, the conventional toxicological testing methods cannot meet this need because they are costly, especially time consuming and less efficient. Here a few examples will be presented on our applications of high throughput and genomic technologies in screening and mechanistic assessment of environmental pollutants.

**287 Determining the molecular toxicological effects of organic flame retardants using an avian *in vitro* screening method** D.E. Crump, Environment Canada / National Wildlife Research Centre, Environment Canada / Canadian Wildlife Service; E. Porter, Environment Canada; S. Chiu, Environment Canada; S.W. Kennedy, Environment Canada, University of Ottawa / Department of Biology, Environment Canada / National Wildlife Research Centre. Risk assessors are challenged with the task of providing data for an increasing number of chemicals. High throughput toxicity screening methods, which permit rapid determination of toxic, molecular and/or biochemical effects of a wide range of chemicals, are essential to help meet this demand. The chicken embryonic hepatocyte (CEH) *in vitro* screening method has been utilized by our laboratory to assess the effects of a wide range of environmental contaminants on (i) cytotoxicity and (ii) mRNA expression of genes associated with xenobiotic metabolism, the thyroid hormone pathway, lipid metabolism and growth. Sixteen structurally variable organic flame retardants (FRs) including, tris(2-butoxyethyl) phosphate, tricresyl phosphate, tetrabromomethylcyclohexane (TBECH) and tris(1-chloropropyl) phosphate (TCPP), were screened using the CEH method in this study. Cytotoxicity data were obtained immediately following the exposure experiment while transcriptional effects, determined via quantitative PCR (QPCR), for 10 target genes were generated in approximately two weeks (from RNA isolation to QPCR data interpretation). Therefore, the screening method is rapid and has the potential to help prioritize chemicals for further assessment. For example, FRs that elicit significant effects on cytotoxicity and/or mRNA expression are prime candidates for egg injection studies that determine adverse effects on the whole animal but are more costly from a time, money and embryo utilization standpoint. The *in vitro* method is also amenable for use with wild avian species (e.g. herring gulls) enabling the determination of (i) effects in species naturally exposed to environmental contaminants and (ii) species differences in sensitivity. This multi-tiered approach – *in vitro* to *in ovo* – to toxicity testing for avian species has been successfully employed for a number of priority environmental contaminants and the screening method offers great promise for FRs and other diverse groups of chemical stressors.

**288 Development of neuromuscular toxic equivalency factors for non-coplanar structures in the rainbow trout (*Oncorhynchus mykiss*)** E.B. Holland Fritsch, I.N. Pessah, University of California- Davis / Molecular Biosciences. Throughout the early part of the 20<sup>th</sup> century polychlorinated biphenyls (PCBs) were used for a number of industrial purposes including a role as plasticizers, adhesives, hydraulic fluid, and flame retardants. Even though they were banned in the 1970's, PCB still remain widely dispersed in the environment and are among the most commonly detected contaminants in fish tissue. Research in fish has focused on toxic effects of coplanar or dioxin-like congeners and their well documented impact on aryl-hydrocarbon receptor (AhR). Therefore, PCBs that effect the AhR are assigned a toxic equivalency factor (TEF) representing their potency as compared to the strongest known AhR activator, 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD). To date little work has focused on the effects of non-coplanar PCBs (ncPCB), or non-dioxin like congeners, which have very little impact on the AhR. Interestingly, studies assessing specific congeners show that ncPCB's are detected in more than 95% of fish tissue from US

lakes accounting for at least 50% of the known PCB load. In mammals, ncPCBs, and other non-coplanar compounds, activate the ryanodine receptor a calcium release channel necessary for excitation-contraction in skeletal and cardiac muscle. Here, ncPCBs cause the receptor to remain in the open state leading to excess calcium release and loss of muscle function. We have utilized similar high-throughput *in vitro* assays to address this impact on rainbow trout skeletal muscle looking at single ncPCBs or environmentally relevant congener mixtures. We have found that PCB95, the most potent ncPCB congener, leads to a 300 fold increase in receptor activity in fish skeletal muscle. The activity of ncPCBs, in relation to PCB95, will be discussed in aim of developing a neuromuscular toxic equivalency for regulatory purposes. Once established these tools can be utilized to address impacts of other non-coplanar structures and combined with TEF matrixes to provide a more complete picture of contaminant effects in the environment.

**289 Multi-tiered Approach to Development of Increased Throughput Assay Models to Assess Endocrine-Disrupting Activity of Chemicals** K.B. Paul, The Hamner Institutes for Health Sciences / Institute for Chemical Safety Sciences, Integrated Systems Toxicology Division / NHEERL, ORD, US EPA, ORISE Postdoctoral Fellow, ORD, US EPA / Integrated Systems Toxicology Division, NHEERL; J.M. Hedge, ORD, US EPA / Integrated Systems Toxicology Division, NHEERL; P.C. Hartig, L.E. Gray, ORD, US EPA / Toxicity Assessment Division, NHEERL; K.M. Crofton, ORD, US EPA / Integrated Systems Toxicology Division, NHEERL; V.S. Wilson, US EPA, ORD, NHEERL, TAD / Reproductive Toxicology Branch, U.S. EPA / Office of Research and Development, ORD, US EPA / Toxicity Assessment Division, NHEERL, U.S. EPA, ORD, NHEERL / Toxicity Assessment Division; S.O. Simmons, ORD, US EPA / Integrated Systems Toxicology Division, NHEERL. Screening for endocrine-disrupting chemicals (EDCs) requires sensitive, scalable assays. Current high-throughput screening (HTPS) approaches for estrogenic and androgenic activity yield rapid results, but many are not sensitive to physiological hormone concentrations, suggesting that less potent EDCs may not be identified. And although thyroid hormone (TH) receptor assays are available, environmental thyroid-disrupting chemicals act primarily via other mechanisms, including inhibition of thyroid hormone synthesis; yet, no HTPS assays exist for TH synthesis targets. We used the following multi-tiered approach to improve assays available for EDC screening: (1) re-optimization of sensitive estrogen and androgen transcriptional activation assays (ER-TA, AR-TA) for HTPS; (2) re-development of an *ex vivo* rat thyroperoxidase (TPO) inhibition assay; and (3) *de novo* development of an *in vitro* model system for human TPO inhibition. We tested the hypothesis that T47D-kbluc ER-TA and MDA-kb2 AR-TA assays could be miniaturized to a 384-well plate, and used controls, dynamic range, variability, and Z' to evaluate this effort. Results demonstrated that the AR-TA assay can be miniaturized without loss of dynamic range (10-fold), increased variability (< 10%), or loss of sensitivity, but that the ER-TA assay could only be automated in 96-well plates due to loss of sensitivity in the 384-well format. Cross-validation of these assays with other ER and AR assays is ongoing with a 90-chemical reference set. Further, we hypothesized that use of a peroxidase substrate (Amplex UltraRed, AUR, LifeTech) and automated reagent delivery could increase the throughput of an *ex vivo* rat TPO assay. The potency of methimazole (MMI)-induced TPO inhibition was similar with AUR compared to the existing guaiacol assay (IC<sub>50</sub> reduced from 5  $\mu$ M to ~1  $\mu$ M), and the AUR signal was stable > 30 min after reaction initiation. Finally, preliminary work demonstrated that lentiviral-transduction of a human cell line (HEK293T) with recombinant TPO could be used to test for human TPO inhibition. Cell fractions showed responsiveness to MMI using AUR as a substrate (IC<sub>100</sub> = 100  $\mu$ M). The results of this step-wise approach to improving and developing assays for EDC screening indicate that systematic evaluation of assay sensitivity and specificity is critical for adaptation of assays to HTPS for EDCs. *This abstract does not necessarily reflect the policy of the US EPA.*

**290 Mechanistic Toxicity Assessment by cellular stress response pathway system ensemble-based Whole-Cell-Array** J. Lan, Northeastern University / Civil & Environmental Engineering; N. GOU, Northeastern University / Civil & Environmental Engineering, Northeastern University / Civil and Environmental Engineering; C. Gao, Northeastern University / Civil and Environmental Engineering; A. GU, Northeastern University / Department of Civil & Env Engineering, Biotechnology Initiative Program, Department of Civil & Env Engineering / Biotechnology Initiative Program.

Toxicological assessment approach for the future (Tox21) demands a paradigm shift from *in vivo* whole animal-based test to *in vitro* mechanistic qualitative biochemical or cell-based assays for rapid, cost-effective toxicological evaluation and characterization of toxic compounds. However, many challenges remain in realizing this transition such as the uncertainty in biomarkers and pathways selection. In recognition of the complexity of the cellular toxic response network, focus on toxicity pathways that result in adverse health outcomes was proposed as Adverse Outcome Pathways (AOP). In this study, we explored and demonstrated the application of cellular stress response pathway ensemble-based toxicogenomics approach for toxicity evaluation of a variety of toxicants. GFP-infused whole-cell array of both *E. coli* and *S. cerevisiae* were employed that allow for real time measurements of the transcriptional and protein expression level activities in exposure to chemicals. Genes/proteins that have been reported to be specific for stress responses in both species were selected and clustered into five stress groups indicative of different types of damage. The 3-D (time, gene, magnitude of change) differential molecular change profiles have sufficient resolution power to discern different chemicals as well as same chemicals at different doses with mechanistic information indicative of their mode of action (MOA). The revealed toxicity mechanisms for the chemicals tested were consistent with their previously known toxic effects. We proposed a new molecular effect level index (TELI for transcriptional level in *E. coli* and PELI for protein expression level in yeast) to quantify the altered gene/protein expression changes, which was shown to exhibit dose-response patterns. Furthermore, the molecular disturbance level-based toxicity endpoints derived from the MELI-based dose response curves were found to correlate with phenotypic endpoints. The demonstration of phenotypic anchoring suggest that, using the stress response pathway ensemble for toxicity assessment is valid and it captures the essential toxic responses that ultimately lead to adverse health outcome, therefore can be applied as a potential method for toxicity assessment.

#### 291 Impacts of Engineered Nanomaterials on Marine Phytoplankton

B. Cole, University of California, Davis / Bodega Marine Laboratory; R. Miller, T. Martin, R. Nesbit, H. Lenihan, University of California, Santa Barbara; G. Cherr, University of California, Davis. Potential impacts of engineered nanomaterials on the marine environment are largely unknown. Determining what these impacts may be is a complicated endeavor that is made more difficult by the nearly limitless diversity in size, shape, and composition of nanomaterials. To determine these impacts we have examined the effects of long-term low-dose exposures of metal and metal oxide nanomaterials on marine phytoplankton. Phytoplankton are fundamental to assessing ocean health as they are the basis of most marine foodwebs, so even subtle changes in growth rates can have large outcomes when scaled up to population or ecosystem levels. Endpoints examined in this study were chosen with the aim of linking cellular targets of toxicity with population and ecosystem level effects so as to provide a broad scale perspective on this issue. We have adapted a suite of fluorescence based assays for cellular targets of cytotoxicity in two species of phytoplankton. These assays can be performed in high-content screens using a fluorescence plate reader, thus facilitating rapid and inexpensive examination of a large number of nanomaterials. Specific cytotoxicity targets include reactive oxygen species production, mitochondrial membrane potential and cell death. Additionally we have determined the population growth rates and decreases in photosystem efficiency in phytoplankton exposed to nanomaterials. These data can be modeled using dynamic energy budgets to predict ecosystem-wide impacts. In combination these assays provide a framework for testing the environmental impacts of nanomaterials on marine phytoplankton across a broad yet interdependent range of scales.

#### 292 High Throughput Bioassays of Urban Stormwater in Combination with AIQS Database GC-MS Screening

M. Allinson, J. Myers, University of Melbourne / CAPIM; V.J. Pettigrove, The University of Melbourne / CAPIM; G. Allinson, Department of Primary Industries, Victoria / Future Farming Systems Research Division; K. Kadokami, University of Kitakyushu. Urban stormwater may contain a large number of organic chemicals, some of which may prove directly toxic to aquatic organisms, while others may elicit more subtle effects, including endocrine disrupting effects. Consequently, stormwater can be a continuing source of water pollution and cause adverse effects to aquatic organisms, directly or indirectly, through immediate exposure, food chain transfer, or through changed prey behaviour or diversity. This pilot study utilised high throughput cytotoxicity test and

hybrid receptor yeast bioassays to assess stormwater quality in five different types of catchments, including industrial and residential areas. The spring and summer in Australia is the most critical time to assess stormwater quality because this is the time of highest demand for recycled water resources, and lowest flow in receiving waters. Grab water samples were collected four times from October 2011 to January 2012 monthly and extracted onto C18 Empore disks after filtration with glass fibre filters. The eluents were split into two and sample non-specific baseline toxicity tested via growth inhibition of *Vibrio fischeri*; the second aliquot was separated into non polar to polar fractions using a Florisil cartridge, and the fractions screened using for a range of yeast-based recombinant receptor-reporter gene bioassays. To investigate the widest possible range of micropollutants, sample extracts were also tested using a gas chromatographic Automated Identification and Quantification System database screening system (GC-MS-AIQS-DB) that can screen samples for more than 900 semi-volatile compounds, including numerous halogenated and non-halogenated hydrocarbons, polynuclear aromatic hydrocarbons (PAHs), polychlorinated biphenyl compounds (PCBs), and range of pharmaceutical and personal care products (PPCPs), and pesticides. The GC-MS-AIQS-DB detected a wide range of chemicals, including herbicides such as simazine, atrazine, and metolachlor, PPCPs such as caffeine and DEET, and the phosphorus based flame retardants, tributyl phosphate and tris(1,3-dichloro-2-propyl) phosphate. The combination of AIQS database screening and bioanalytical tools proved a very useful method to assess stormwater quality, and appeared to contain the inherent periodic chemical variability of this water resource.

#### 293 Combining *in vitro* toxicity data with QSARs and mass balance exposure models for high throughput risk assessment

J.A. Arnot, ARC Arnot Research & Consulting Inc., University of Toronto Scarborough / Department of Physical and Environmental Sciences; T.N. Brown, Helmholtz Centre for Environmental Research UFZ / Department of Analytical Environmental Chemistry, Helmholtz Centre for Environmental Research / Department of Analytical Environmental Chemistry, Helmholtz centre for environmental research – UFZ / Department of Analytical Environmental Chemistry; K. Breivik, Norwegian Inst. for Air Research; R.S. Thomas, The Hamner Institutes for Health Sciences / Institute for Chemical Safety Sciences, Center for Genomic Biology and Bioinformatics; B.A. Wetmore, The Hamner Institutes for Health Sciences. Few measured exposure and toxicity data are available for the majority of the thousands of chemicals produced and used in society. Evaluating the potential human health and environmental risks for all chemicals using traditional monitoring and animal testing data is not feasible. *In vitro* and *in silico* methods provide plausible alternatives for screening level, high throughput risk assessment (HTRA) (i) to prioritize existing chemicals for more comprehensive evaluation, and (ii) to provide guidance for the design of new and replacement chemicals. Due to data gaps in chemical information, particularly for chemical usage and release rates, estimated values and Quantitative Structure-Activity Relationships (QSARs) are required resulting in considerable assessment uncertainty. Here, we combine recently developed strategies in *in vitro*, *in silico*, emission rate estimation, and screening level uncertainty methods for HTRA. The Risk Assessment, IDentification And Ranking (RAIDAR) mass balance model combines information on chemical emissions, partitioning, degradation rate, environmental fate, food web bioaccumulation, far-field human exposure, and toxicity in an evaluative framework. The model quantitatively links chemical emissions and internal doses and intake rates corresponding to effects. In this study, *in vitro* high-throughput screening data and pharmacokinetic (PK) models are used to estimate intake rates and internal concentrations (*i.e.* estimated *in vivo* doses) associated with the biological pathway altering dose (BPAD) in humans and to parameterize RAIDAR to back-calculate the “critical” emission rate corresponding to the BPAD. The calculated critical emission rates include the propagation of uncertainty in BPAD estimates and in chemical partitioning properties and degradation rates that are also used to parameterize the model. Calculated distributions for the critical emission rates are then compared with estimates of actual emission rates (also including uncertainty estimates) to provide an indication of the likelihood for potential risk. The *in vitro-in silico* HTRA framework thus links chemical emissions with *in vitro* biological activity including the propagation of uncertainty in key information used in chemical assessments in the model calculations. The method is applied as a case study to chemicals that are part of the U.S. Environmental Protection Agency's ToxCast program™. Future improvements to the methods are discussed.



#### 294 Using isotopically modified silver nanoparticles to assess their bioavailability and toxicity at environmentally realistic exposures M. Croteau, U.S. Geological Survey; A. Dybowska, Natural History Museum London / Mineralogy; S.N. Luoma, University of California Davis / John Muir Institute of the Environment, U.S. Geological Survey, University of California, Davis; E. Valsami-Jones, University of Birmingham. One challenge in understanding the environmental implications of nanotechnology lies in tracing nanoparticles in organisms during controlled experimental studies as well as in the environment. Tracers are usually required because the natural background metal concentrations limit the ability to detect bioaccumulation at environmentally relevant exposures. Stable isotope methodologies offer great potential to address these challenges as metallo-nanoparticles can be synthesized from a metal enriched with a rare stable isotope. For example, we demonstrate that newly accumulated Ag concentrations as low as 0.05 nmol g<sup>-1</sup> can be detected in freshwater snails exposed to waterborne Ag concentration as low as 0.1 nM, if isotopically enriched is used (e.g., <sup>109</sup>Ag enriched at 99.7%). Exposures more than 20-times higher would otherwise be required to detect Ag uptake if no tracer were used. Without a tracer, Ag uptake rates can only be determined at concentrations typical of extreme contamination. Here we use a stable isotope methodology to quantify the bioavailability of silver nanoparticles (AgNPs) from food and water under conditions that might be typical of nature, and develop links to toxicity. Citrate-capped Ag NPs were synthesized using enriched <sup>109</sup>Ag isotope. The tracing technique allows determining uptake rate constant for freshwater snails exposed to AgNPs either dispersed in water at concentrations ranging from 0.1 to 30 nM, or mixed with food at dietborne concentrations ranging from 1 to 200 nmoles per gram. The isotope tracer methodology was also used to quantitatively evaluate physiological processes like feeding rates and assimilation efficiency that might be adversely affected by exposure to AgNPs in diet. Along with distribution coefficients, the rate constants developed in these experiments were used to model Ag bioaccumulation from both routes under environmentally realistic conditions.

Without a tracer, Ag uptake rates can only be determined at concentrations typical of extreme contamination. Here we use a stable isotope methodology to quantify the bioavailability of silver nanoparticles (AgNPs) from food and water under conditions that might be typical of nature, and develop links to toxicity. Citrate-capped Ag NPs were synthesized using enriched <sup>109</sup>Ag isotope. The tracing technique allows determining uptake rate constant for freshwater snails exposed to AgNPs either dispersed in water at concentrations ranging from 0.1 to 30 nM, or mixed with food at dietborne concentrations ranging from 1 to 200 nmoles per gram. The isotope tracer methodology was also used to quantitatively evaluate physiological processes like feeding rates and assimilation efficiency that might be adversely affected by exposure to AgNPs in diet. Along with distribution coefficients, the rate constants developed in these experiments were used to model Ag bioaccumulation from both routes under environmentally realistic conditions.

#### 295 Impact of Nano-Ceria Fuel Additive on Physicochemical and Toxicological Properties of Diesel Exhaust Particles J. Zhang, Div of Env Health, Keck School of Medicine, University of Southern California; L. Zhang, Env and Occup Health Sciences Institute; G. Mainelis, Y. Nazarenko, L. Calderon, Dept Env Sciences, Rutgers University; P.J. Liroy, Env and Occup Health Sciences Institute; S. Sarkar, S. Schwander, UMDNJ-School of Public Health, Dept of ENOH; W. Baker, J. Seiffert, A.J. Thorley, T.D. Tetley, K. Chung, National Heart and Lung Institute, Imperial College London; P. Subramaniam, K. Lee, E. Garfunkel, Dept of Chemistry and Chemical Biology, Rutgers University; S. Chen, M. Ryan, A. Porter, Dept Materials and London Centre for Nanotechnology, Imperial College London; H. Kipen, UMDNJ-Robert Wood Johnson Medical School; H. Solomon, Div of Env Health, Keck School of Medicine, University of Southern California. As a catalyst to promote combustion efficiency, ceria (CeO<sub>2</sub>) nanoparticles, have been increasingly used in Europe and elsewhere as diesel fuel additive. Although the use of ceria additive leads to increased fuel combustion efficiency and reduced particle mass emissions, it remains unknown if the additive would affect physicochemical and toxicological properties of the exhaust. We conducted a series of experiments to evaluate the impact of a commercially available ceria-based diesel additive, Envirox™ (Energenics Ltd., UK) at various ceria concentrations in a regular ultra-low sulfur diesel fuel, ranging from 0 to 10 times the manufacturer's recommendation. Using a one-cylinder electrical generator (Yanmar) under 100% load, we directed a portion of the exhaust into a stainless steel testing chamber where gaseous constituents (CO, CO<sub>2</sub>, NO<sub>x</sub>, and aldehydes) and particulate components (particle size, mass concentration, and number concentration) were characterized. Exhaust particles were also collected onto filters to analyze their chemical composition (e.g. elemental carbon, organic carbon, elements, and PAHs), charge (zeta potential) and toxicity. We observed that with increasing CeO<sub>2</sub> concentrations in the fuel: (1) the total energy output per liter of fuel (kW/liter) increased as expected for a combustion catalyst; (2) the total exhaust particle number concentration increased per particles mass basis, (3) the median particle diameter decreased, (4) the ratio of particle mass concentration to CO<sub>2</sub> concentration decreased, (5) total PAH mass content in exhaust particles decreased, (6) elemental carbon and total carbon mass content in exhaust particles decreased, respectively, (8) NO<sub>x</sub> concentration increased per particle mass basis, and (7) the zeta potential of exhaust particles decreased. Preliminary data also suggests a change in the toxicological response of the particles. Using *in vitro* cell culture studies,

we observed that with increasing CeO<sub>2</sub> concentrations in the fuel, exhaust particles exhibited *decreased* induction of inflammatory cytokine mRNA and *decreased* production of TNF-α, IL-1β proteins in THP-1 cells. To further examine toxicological impacts of the CeO<sub>2</sub> additive, we are currently conducting additional *in vitro* tests using other cell types and endpoints. These changes in toxicity will be discussed in the context of the variation in the physicochemical properties of exhaust particles.

#### 296 Diesel Exhaust Particle and DEPenvirox Effects on Protein and mRNA Expression in Stimulated THP-1 and Primary Human Peripheral Blood Mononuclear Cells S. Schwander, S. Sarkar, UMDNJ-School of Public Health, Dept of ENOH; J. Zhang, Div of Env Health, Keck School of Medicine, University of Southern California; L. Zhang, Env and Occup Health Sciences Institute; W. Baker, J. Seiffert, National Heart and Lung Institute, Imperial College London; M. Ryan, A. Porter, Dept Materials and London Centre for Nanotechnology, Imperial College London; K. Lee, Dept of Chemistry and Chemical Biology, Rutgers University; A.J. Thorley, T.D. Tetley, K. Chung, National Heart and Lung Institute, Imperial College London. Correlations between physicochemical characteristics of freshly collected DEP from conventional diesel fuel sources and diesel fuel supplemented with cerium oxide-containing Envirox additive (DEP<sub>envirox</sub>) (see abstract from J. Zhang) and biological responses in *M. tuberculosis*-stimulated human immune cells have not been studied in detail. As part of our *in vitro* studies of cellular, molecular and immune reactivity, we set out to assess the effects of DEP and DEP<sub>envirox</sub> in the human monocytic leukemia cell line THP-1, in human peripheral blood mononuclear cells (PBMC) and human alveolar lung cells from healthy adult donors. Cells were exposed to DEP and DEP<sub>envirox</sub> or left untreated (control) for 4 and 24 hrs followed by extraction of total RNA. THP-1 cells were also stimulated with LPS (100ng/mL) as a positive control for gene expression. cDNA was then generated and mRNA expression assessed using gene specific primers by Real-time PCR with Power SYBR green PCR master mix in a ABI 7900HT. Data were analyzed by the relative quantitation method (DDCt method). Abundance of specific mRNAs in MNP exposed THP-1 cells was determined relative to untreated control THP-1 cells. Exposure of THP-1 cells and PBMC to DEP led to a dose-dependent increase of expression of mRNAs encoding *TNFA*, *IL1b*, *IL6*, and *IL12p40*. Interestingly, this DEP-mediated increase of inflammatory cytokine mRNA expression was not observed in cells exposed to DEP<sub>envirox</sub>. Similarly, the production of TNF-α and IL-1β proteins was increased in a dose-dependent fashion in presence of DEP but not in presence of DEP<sub>envirox</sub> in THP1 cells. Thus, the exposure of THP-1 cells to DEP and DEP<sub>envirox</sub> led to clearly distinguishable activation and gene expression patterns. DEP<sub>envirox</sub> obtained from the combustion of diesel fuel containing increasing proportions of Envirox™ additive (0.1x, 1.0x, 10x) was correlated with further reduced induction of inflammatory cytokine mRNA and protein production in THP-1 cells. We currently examine the effects of DEP and DEP<sub>envirox</sub> from another collection in our CEF in additional experiments to confirm our observations. These studies are also being extended to examine the bioreactivity of DEP and DEP<sub>envirox</sub> using *in vitro* models of human alveolar epithelial cells. Nevertheless, our data suggest that diesel additive Envirox™ may alter the physicochemical properties typical for DEP with a resulting decreased immunotoxicity.

we observed that with increasing CeO<sub>2</sub> concentrations in the fuel, exhaust particles exhibited *decreased* induction of inflammatory cytokine mRNA and *decreased* production of TNF-α, IL-1β proteins in THP-1 cells. To further examine toxicological impacts of the CeO<sub>2</sub> additive, we are currently conducting additional *in vitro* tests using other cell types and endpoints. These changes in toxicity will be discussed in the context of the variation in the physicochemical properties of exhaust particles.

#### 297 Potential Consumer Exposure to Airborne Ag and Zn Nanoparticles due to the use of Nanotechnology-enabled Consumer Sprays G. Mainelis, L. Calderon, Y. Nazarenko, Dept Env Sciences, Rutgers University; K. Lee, E. Garfunkel, Dept of Chemistry and Chemical Biology, Rutgers University; S. Schwander, UMDNJ-School of Public Health, Dept of ENOH; T.D. Tetley, National Heart and Lung Institute, Imperial College London; P.J. Liroy, Env and Occup Health Sciences Institute; K. Chung, National Heart and Lung Institute, Imperial College London; J. Zhang, Div of Env Health, Keck School of Medicine, University of Southern California. Engineered nanomaterials are increasingly applied in consumer products; however, potential exposures to nanoparticles and the resulting adverse health effects have been only minimally explored. We investigated potential consumer exposures to the use of sprays designed for cleaning, odor control, and sun protection containing either silver or zinc nanoparticles. We chose to study silver or zinc nanoparticles because they were shown to induce toxic changes in respiratory epithelial cells and immune cells. The potential size range of particles released during spray use was investigated by aerosolizing each product in a testing chamber using Collison nebulizer (BGI, Inc.) and C-flow (Saville, Inc.) atomizer, and a sprayer supplied with each product.

we observed that with increasing CeO<sub>2</sub> concentrations in the fuel, exhaust particles exhibited *decreased* induction of inflammatory cytokine mRNA and *decreased* production of TNF-α, IL-1β proteins in THP-1 cells. To further examine toxicological impacts of the CeO<sub>2</sub> additive, we are currently conducting additional *in vitro* tests using other cell types and endpoints. These changes in toxicity will be discussed in the context of the variation in the physicochemical properties of exhaust particles.



Size distribution and concentration of the released particles were measured using a Scanning Mobility Particle Sizer and an Aerodynamic Particle Sizer (models 3080 and 3321, TSI, Inc., Shoreview, MN). The cleaning and odor control products were then realistically used in a simulated home environment while measuring the user's personal exposure. The use of sun protection products was simulated using a human mannequin while sampling through its nose. Air samples were taken to investigate sizes, shapes and agglomeration of the released particles using Transmission Electron Microscopy and other techniques. When tested in the chamber, the products released particles not only in the nanosize range ( $< 100$  nm) but, interestingly enough, in coarse (2.5-10  $\mu\text{m}$ ) and for some products in super-coarse ( $>10\mu\text{m}$ ) particle size ranges. The particle number concentration depended on the product and ranged from  $10^4$  to  $10^6$  particles/ $\text{cm}^3$  with the mass of particulate matter being in tens of  $\mu\text{g}/\text{m}^3$ . When used in the simulated home environment, potential exposures to nanosized, micron-sized, and super-micron sized particles were observed as well. The TEM analysis confirmed that for most of the products, the released particles included nanoparticles, their agglomerates as well as nanosized particles attached to larger particles. The release and inhalation of nanoparticles and their agglomerates in such a wide size range would result in particle deposition in all regions of the respiratory system and thus, health studies should focus not only on single nanoparticles, but also on deposition and health effects of larger agglomerates. The next steps of the study include *in vitro* and *in vivo* investigation.

### 298 Bioreactivity of Zinc Oxide and Silver Nanoparticles with the Human Lung Respiratory Unit and Human Monocytic Cells T.D. Tetley,

W. Baker, A.J. Thorley, J. Seiffert, K. Chung, National Heart and Lung Institute, Imperial College London; J. Zhang, Div of Env Health, Keck School of Medicine, University of Southern California; A. Porter, M. Ryan, Dept Materials and London Centre for Nanotechnology, Imperial College London; S. Sarkar, S. Schwander, UMDNJ-School of Public Health, Dept of ENOH. These studies aim to determine the effect of nanoparticles (NP) on the alveolar unit. Alveolar type I (AT1) epithelium accounts for 95% of the alveolar surface and are important target cells. We have shown that nanoparticles can translocate across the alveolar epithelium, to access the interstitial compartment (where fibroblasts reside, being important in maintaining structure and in repair) and may then access the vasculature. Immortalized human ATI cells and primary human lung fibroblasts were exposed to ZnO and silver (Ag; 1-50mg/ml) for 4 and 24h and assessed for cytotoxicity and bioreactivity. At 4h polydisperse ZnO induced dose-related cell death, viability falling to 60%, then 50%, of control at 10 and 50mg/ml. By 24h, viability fell to only 10% of control at  $>25\text{mg}/\text{ml}$  ZnO. LDH release increased with increased cell death. Oxidative cell stress increased with ZnO dose at 4h, but by 24h could not be detected. ZnO caused a similar loss of cell viability in fibroblasts at 4h, with no further change at 24h. However, at the high doses of ZnO, there was marked fibroblast LDH release at both time intervals, suggesting that, whilst AT1 cells do not recover from ZnO-induced cell death, fibroblasts proliferate to replace damaged cells. These responses will be compared to ZnO nanowires, which have now been synthesized for these studies. AT1 cells were exposed to 10 and 50nm Ag spheres, and Ag nanowires. There was a small but consistent fall in AT1 cell viability at 24h, to between 80-90% of control, following exposure to  $>10\text{mg}/\text{ml}$  of all types of Ag. Bioreactivity was measured as CXCL8 (IL-8; attracts neutrophils) and CCL-2 (MCP-1; attracts monocytes) release. Although 5mg/ml of 10nm Ag had no effect on CXCL8, 25mg/ml caused a 12-fold increase. 50nm Ag caused a 3-fold and 5-fold increase in CXCL8 at 5 and 25mg/ml. In contrast, Ag nanowires did not affect CXCL8 release. Ag either had no effect (50nm), or inhibited CCL2. Thus, 25mg/ml 10nm spheres inhibited CCL2 to 40%, while 5 and 25mg/ml of silver nanowires inhibited CCL2 release to 50% and 25%, respectively, of control levels. Thus, Ag spheres stimulated CXCL8, while 10nm spheres inhibited CCL2; nanowires did not affect CXCL8, and inhibited CCL2. Therefore, responses to ZnO and Ag nanoparticles depend on structure, chemistry and target cell. These studies are being extended to other human alveolar cells and human monocytic cells, THP1 and monocyte-derived macrophages, before and after infection with M tb.

### 299 The Biostability and Immunogenic Effects of Silver Nanostructures in Simulated Body Fluids (SBFs) and Human Epithelial and Blood Cells M. Ryan, A. Porter, S. Chen, I. Theodorou, A. Goode, B.F. Leo, E. McGuire, Dept Materials and London Centre for Nanotechnology, Imperial College London; M. Shaffer, Dept. Chemistry and London Centre for

Nanotechnology, Imperial College London; A.J. Thorley, T.D. Tetley, K. Chung, National Heart and Lung Institute, Imperial College London; S. Sarkar, S. Schwander, UMDNJ-School of Public Health, Dept of ENOH; J. Zhang, Div of Env Health, Keck School of Medicine, University of Southern California. Ag nanoparticles are in wide-spread use in consumer products such as wound-dressings, anti-bacterial sprays, odor-control fabrics and in a whole range of electrical systems which release Ag nanoparticles to impart some 'hygienic' function (for example in washing machines and hair-dryers). The market for nano-Ag products is already significant and growing rapidly; the potential for individual exposure of airborne Ag-nanoparticles by inhalation is therefore high. The toxicity of metallic and metal oxide nanomaterials has been linked to their dissolution behaviour. The aim of this project is to assess whether, and by which mechanism, the physiological properties (*i.e.* aggregation state, morphology and chemistry) of the Ag nanostructures (both wires and particles) change in biofluids and human cells (lung epithelial, THP-1 and PBMC cells). Using a combination of *in-situ* scattering and spectroscopic methods, we have demonstrated that the dissolution rate of the silver nanoparticles increases with decreasing pH and is size-dependent. The Ag nanoparticles dissolved more rapidly in a simulated body fluid of lysosomal pH, whereas they were comparatively stable at extracellular pH. The silver particles dissolved *inside* epithelial cells and their destination inside the cell became modified when they were incubated in lung surfactant prior to cell exposure. We will discuss visualization of the degrading nanowires inside the cells using energy filtered transmission electron microscopy (EFTEM) in combination with electron energy loss spectroscopy (EELS) to assess the morphological changes observed during intracellular dissolution. The implications of silver dissolution on cell metabolism and lung function will be discussed.

### 300 Characterisation of exposure and effects of silver nanoparticles to the earthworm *Lumbricus rubellus* N. Van den Brink, Alterra / Dept. of

Ecology & Environment; M. van der Ploeg, Alterra, Wageningen UR; H. van den Berg, Wageningen University / Division of Toxicology; R. Handy, University of Plymouth / School of Biomedical and Biological Sciences; R. Peters, RIKILT, Wageningen University / Residues & Contaminants, RIKILT / Contaminants; P. Waalewijn-Kool, VU University / Department of Ecological Science; G. Koopmans, B. Molleman, Wageningen University / Sub-department of Soil Quality; I. Rietjens, Wageningen University / Division of Toxicology. Risks of engineered nanoparticles are under debate, and further scientific underpinning of the assessment of these risks is still needed. Silver nanoparticles (Ag-NPs) are used widely, and relatively many studies have investigated hazards that these particles may pose to organisms. However, up till now relatively little attention has been paid to soil organisms. Literature shows that effects of Ag-NPs on earthworms depend on, among others, soil type, particle size and the surface coating of the NPs (Shoults-Wilson et al 2011a,b). In order to elucidate the hazards of non-coated Ag-NPs to earthworms, we exposed earthworms (*Lumbricus rubellus*) to non-coated Ag-NPs (NM-300K in Tween20,  $< 20$  nm, Fraunhofer). Adult earthworms were exposed for four weeks, after which growth and reproduction was assessed (cf. OECD 222). Animals were exposed to Ag-NPs at 0, 1.54, 15.4 and 154 mg/kg soil and to  $\text{AgNO}_3$  as a ionic control (15.4 mg/kg). F1 animals were further exposed to the same levels to which their parents were exposed and effects on stage dependent growth and mortality were quantified. The Ag-NPs in spiking extract and the exposure during the experiments were characterised by using AF4 in combination with UV and ICP-MS, single-particle ICP-MS, Atomic Absorption Spectrometry (AAS) and SEM-EDX. Extensive exposure characterisation showed the occurrence of primary particles in soil extracts, which were approximately 16 nm in size, but also the presence of larger agglomerates was detected. Furthermore, in contrast to Ag-NPs in the spiking solution, Ag-NPs in soils showed traces of Cl, indicating possible deposition of AgCl on the surface of the Ag-NPs. Ag-NPs in the soil with the highest levels showed release of Ag ions in the soil pore water, even after 6 months of incubation. The lower Ag-NP and  $\text{AgNO}_3$  treatments of the soil showed only low ionic concentrations in the pore water after 6 months. Effects of Ag-NP treatment were evident at different levels of biological integration. Juvenile mortality was 100% at the highest Ag-NP dose, adult growth and cocoon production were also affected at this concentration. Effects of  $\text{AgNO}_3$  were visible at 15.4 mg/kg. In the presentation, more details will be given on the effects of Ag-NPs on histological endpoints and on the population growth rate. These effects will be discussed in relation to the exposure characterisation to elucidate whether

Ag-NP toxicity may just be caused by ionic exposure or also through NP-properties.

**301 Interactions of Humic and Fulvic Acids with Silver Nanoparticles and the Resultant Toxicity to *Chlamydomonas Reinhardtii*** R.M. Sofield, Western Washington University / Huxley College of Environment; B. Wagner, Eawag; L. Sigg, Eawag, Eawag, Swiss Federal Institute of Aquatic Science and Technology / Environmental Toxicology; R. Behra, Eawag / Environmental Toxicology. It is generally accepted that the free ion form of most metals is the toxic form, but this concept was not developed with consideration of the unique characteristics of nanoparticles. For example, with silver nanoparticles (AgNPs) in solution the Ag can exist as both elemental silver and Ag<sup>+</sup>. When natural organic matter (NOM) is present, it may complex the Ag<sup>+</sup> or interact with the AgNP, potentially affecting the rate of Ag oxidation; this suggests that the kinetics of ion release are essential to consider with metals based nanoparticles. Finally, many AgNPs have capping agents, which may themselves alter the chemical fate, transport, and toxicity. The goals of this study were to evaluate the chemical complexation and toxicity to *Chlamydomonas reinhardtii* of Citrate, CO<sub>3</sub>, and PVP capped AgNPs and AgNO<sub>3</sub> in MOPS buffer only and in MOPS with 8 mg/L Suwannee River humic or fulvic acids (representative of NOM). The Ag was pre-equilibrated in the appropriate solution at room temperature in the dark for 24 hours. Polymer based Ion Selective Electrodes were then used to determine the concentration of dissolved Ag<sup>+</sup> for each AgNP or AgNO<sub>3</sub> solution. The photosynthetic effects (IC50s) to *C. reinhardtii* were also determined in the MOPS solutions. The toxicity ranking of the capped AgNPs in MOPS only was PVP > Citrate > CO<sub>3</sub>. In the presence of both humic and fulvic acids, the toxicity of all AgNPs and AgNO<sub>3</sub> as total Ag decreased. Further work is being conducted to confirm the concentrations of dissolved Ag<sup>+</sup> for each of the AgNPs and AgNO<sub>3</sub> solutions. This will be compared to the toxicity results based on total Ag.

**302 Identification of novel polyfluorinated compounds in natural waters using accurate mass TOFMS** M.J. Strynar, US Environmental Protection Agency / National Exposure Research Laboratory, U.S. EPA / HEASD, U.S. EPA / NERL/HEASD; S. Dagnino, IRCM Institut de Recherche en Cancérologie de Montpellier, INSERM U896; A.B. Lindstrom, U.S. Environmental Protection Agency / National Exposure Research Laboratory, U.S. EPA; E.M. Andersen, U.S. Environmental Protection Agency / US EPA; L. McMillan, National Caucus & Center on Black Aged, Inc.; M. Thurman, University of Colorado in Boulder / Center for Environmental Mass Spectrometry; I. Ferrer, Center for Environmental Mass Spectrometry, University of CO – Boulder / Civil, Environmental and Architectural Engineering; C. Ball, Agilent Technologies, Inc.. Perfluorinated compounds (PFCs) have been detected in a multitude of environmental and biological matrices across the globe for a number of years. This scientific scrutiny and concerns over exposure, toxicity and risk has led to international regulatory efforts that have resulted in the reduction or elimination of certain perfluorinated compounds in various products and waste streams in recent years. In addition, a few manufacturers have been moving toward the production of shorter chain per- and polyfluorinated compounds in order to reduce the potential for bioaccumulation in humans and wildlife. Some manufacturers are producing polyfluorinated compounds that contain central ether oxygens in order to avoid traditional perfluorinated structures. At present, there has been very limited information published on these "replacement chemistries" in the peer reviewed literature. Using an Agilent 6200 series accurate mass LC/MSD TOF we were able to identify novel polyfluorinated compounds in natural water samples taken from locations with historical perfluorinated compound contamination. Identification of these novel compounds includes accurate mass fragments from in-source collision-induced dissociation (CID), evaluation of adducts formed during analysis, and confirmation with authentic standards when possible. An additional line of evidence that is often seen is a homologous series of similar compounds that differ by multiples of CF<sub>2</sub> ( $m/z = 50$ ). These related compounds have logical chromatographic separation from the major target compound and they also have comparable fragments and adducts produced during analysis. A key element to this type of evaluation is the manipulation of the fragmenter voltage used in the TOF system in order to form both ionized parent molecules and a logical series of diagnostic fragment ions that help elucidate structures. This presentation will focus on TOF-based analytical approaches that can be used to quantify traditional PFCs from areas with known historical

contamination and to identify new per- and polyfluorinated materials that have not yet been described in the literature.

**303 Are all N-alkyl substituted perfluorooctane sulfonamides PFOS-precursors? The case of SAmPAP diester biodegradation in marine sediments** J.P. Benskin, AXYS Analytical Services Ltd; M.G. Ikonomou, Institute of Ocean Sciences / Department of Fisheries and Ocean; F.A. Gobas, Simon Fraser University / School of Resource and Environmental Management (Faculty of Environment), Simon Fraser University / School of Resource & Environmental Management, Faculty of Environment, Simon Fraser University; M.B. Woudneh, AXYS Analytical Services Ltd / R&D Chemist; T. Begley, Food and Drug Administration / Center for Food Safety and Applied Nutrition; J.R. Cosgrove, AXYS Analytical Services Ltd. N-ethyl perfluorooctane sulfonamidoethanol-based phosphate (SAmPAP) diester is a commercial surfactant which was widely used in the food paper and packaging industry from the 1970s to 2002 and continues to be manufactured in Asia. SAmPAP diester was recently measured for the first time in urban marine sediments, but little is known about its stability in this environment. In the present work, the biodegradation of SAmPAP diester and N-ethyl perfluorooctane sulfonamidoethanol (EtFOSE) by bacteria in marine sediments was examined over 120 days at 4°C and 25°C. At both temperatures, EtFOSE was transformed to a suite of products, including N-ethyl perfluorooctane sulfonamidoacetate, perfluorooctane sulfonamidoacetate, N-ethyl perfluorooctane sulfonamide, perfluorooctane sulfonamide and perfluorooctane sulfonate. Transformation was significantly more rapid at 25°C compared to 4°C. In contrast to EtFOSE, SAmPAP diester was highly recalcitrant to microbial degradation, with negligible loss and/or product formation observed after 120 days at both temperatures. We hypothesize that the hydrophobicity of SAmPAP diester reduces its bioavailability, thus hindering microbial biotransformation in sediments. The resistance of SAmPAP diester to biodegradation may explain why this substance is still observable in marine sediments over a decade after its phase-out in North America.

**304 Analysis of Chlorinated and Fluorinated Contaminants Formed in Thermal Degradation of Halogenated Polymers** A.L. Myers, University of Toronto / Chemistry; S.A. Mabury, University of Toronto / Department of Chemistry; K.J. Jobst, E.J. Reiner, Ontario Ministry of the Environment. Chlorine and fluorine are incorporated into commercial thermoplastics, such as poly(vinyl chloride) (PVC), poly(vinylidene fluoride) (PVDF), poly(chlorotrifluoroethylene) (PCTFE) and poly(tetrafluoroethylene) (PTFE) to increase the thermal stability of the polymer. Nevertheless, these polymers have been shown to thermally degrade to form persistent and toxic halogenated environmental contaminants. This is a result of the eventual fate of these compounds, which includes thermal degradation through waste incineration, recycling, accidental fire, or backyard barrel burning. In this study, we have investigated the formation of chlorinated and fluorinated contaminants through thermal degradation of PVC, PVDF, PCTFE, and PTFE in a quartz tube furnace. The identification of the resulting degradation products was achieved by Fourier transform ion cyclotron resonance mass spectrometry (FTICR-MS), as well as by gas chromatography mass spectrometry (GC-MS), and liquid chromatography tandem mass spectrometry (LC-MS/MS). Our preliminary results indicate that a wide range of halogenated compounds, including perhalogenated-carboxylic acids, are generated in the thermolysis experiments. This study also shows that the degree and type of polymer halogenation determines the structural nature of the thermal degradation products.

**305 Identification of Novel Fluorochemicals in Aqueous Film-Forming Foams (AFFF) Used by the US Military** B. Place, Oregon State University / Department of Chemistry; J. Field, Oregon State University / Department of Environmental and Molecular Toxicology. Aqueous Film-Forming Foams (AFFF) are chemical mixtures that are used by military and non-military organizations to extinguish hydrocarbon-based fires (such as aircraft crashes and petroleum fires). Historical releases of AFFF (both controlled and uncontrolled) have been reported and previous studies identified high concentrations of commonly studied fluorochemical surfactants (such as PFOA and PFOS) in environmental compartments adjacent to these AFFF spills. The difficulty in fully understanding the environmental impact of the release of AFFF is that most AFFF products are mixtures of proprietary fluorochemicals. An analytical method was developed to identify the elemental composition of multiple fluorochemicals in AFFF. The method utilized two

mass spectrometric tools for this purpose: fast atom bombardment mass spectrometry and high resolution mass spectrometry (via quadrupole-time-of-flight mass spectrometer analysis). In addition, patent-based structural information was combined with mass spectrometry to elucidate the chemical structure of the fluorochemicals. US military-approved AFFF products were sampled from twenty different military bases across the US, representing all military AFFF manufacturers and covering a wide range of AFFF manufacturing dates. Eleven different fluorochemical classes were identified as primary components in AFFF from seven different formulators. The fluorochemical classes determined include surfactants with anionic, cationic and zwitterionic functionalities and with fluorinated chains lengths from 4 to 10. Of these identified components, ten of the classes have minimal peer-reviewed studies related to the environmental presence and/or toxicity.

**306 Identification of biomarkers of exposure to PAPs and FTOHs in urine and serum with LC-time-of-flight-MS** S. Dagnino, IRCM Institut de Recherche en Cancérologie de Montpellier, INSERM U896; A.B. Lindstrom, U.S. Environmental Protection Agency / National Exposure Research Laboratory; L. Mc Millan, E.M. Andersen, U.S. Environmental Protection Agency; C. Ball, Agilent Technologies, Inc.; C. Lau, US EPA / Toxicity Assessment Division; M.J. Strynar, US Environmental Protection Agency / National Exposure Research Laboratory, U.S. EPA / HEASD, U.S. EPA / NERL/HEASD. Perfluorinated compounds (PFCs), such as perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA), have become the subject of intense investigation and growing regulatory control in the USA and the EU. In contrast, little is known about many of the more complex or newly formulated PFCs, despite the fact that they are used in a wide range of consumer products and human exposure routes remain poorly described. Some of the less well-studied compounds include the fluorotelomer alcohols (FTOHs) and polyfluoroalkyl phosphate esters (PAPs) both of which are used in numerous applications such as oil and water repellent treatments for carpets and coatings for food packaging. Although their toxicity has yet to be clearly determined, FTOHs and PAPs are known to degrade to terminal perfluorinated carboxylic acids (PFCAs) through metabolic pathways that produce a number of toxic reactive intermediates. Therefore, it is important to characterize exposures for FTOHs and PAPs, and to elucidate their complex metabolism *in vivo*. The first aim of this study was to identify FTOH and PAP metabolites in a rodent model, and then to determine if these compounds can be used as biomarkers for human exposure research. A sensitive liquid chromatography-electrospray ionization time-of-flight mass spectrometry method was established for the identification of urinary and serum metabolites of 8:2 FTOH and the 8:2, 8:2 diPAP in adult Sprague-Dawley male rats dosed at 5 mg/kg and 50 mg/kg. Resulting PFOA levels were as high as 1500 ng/mL in serum and 600 ng/mL in urine of FTOH and PAP dosed rats. Unique and specific biomarkers were also detected in these test animals and correlations between urine and serum concentrations have been established. With the application of these methods to anonymous adult human serum and urine samples (n = 100), previously reported and new per- and poly-fluorinated metabolites were identified. This work has led to the tentative identification of biomarkers of exposure for FTOHs and PAPs in humans while also demonstrating the potential utility of urine-based biomarkers as a non-invasive approach for human studies.

**307 Time-resolved global emission inventories for C4-C14 perfluoroalkyl carboxylic acid homologues from 1951 to 2050** Z. Wang, Swiss Federal Institute of Technology (ETH Zurich) / Institute for Chemical and Bioengineering; I.T. Cousins, Stockholm University; M. Scheringer, Swiss Federal Institute of Technology (ETH Zurich) / Institute for Chemical and Bioengineering; R.C. Buck, E. I. duPont de Nemours & Co., Inc. / DuPont Chemicals & Fluoroproducts, DuPont / Chemicals & Fluoroproducts, E. I. duPont de Nemours & Co., Inc. / Chemicals & Fluoroproducts; K. Hungerbuehler, Swiss Federal Institute of Technology (ETH Zurich) / Institute for Chemical and Bioengineering. For over a decade, long-chain perfluoroalkyl carboxylic acids (PFCAs,  $C_xF_{2x+1}COOH$ ,  $x = 7-13$ ), including perfluorooctanoic acid (PFOA) and perfluorononanoic acid (PFNA), have attracted attention as global contaminants of high concern due to their persistence, bioaccumulative potential and global presence in the abiotic environment, biota and humans. To fully characterize the exposure to PFCAs, an understanding of their cycling in the environment, starting from source identification and emission quantification, is needed. In 2006, Prevedouros et al. estimated the total global historical emissions of PFCAs (1951-2004) and highlighted the significance of direct industrial sources to the presence

and distribution of PFCAs in the environment. However, their work focused mainly on PFOA and PFNA, and a detailed breakdown of PFCA sources on a homologue basis was not provided. In addition, industry has taken action to replace the longer-chain homologues with compounds containing shorter perfluorinated chains in recent years, suggesting that potential emission sources for PFCAs have changed since the review was published. Moreover, new manufacturers have begun to make long-chain PFCAs and their potential precursors. In the current study, we re-evaluated and expanded the work of Prevedouros et al. in the following ways, namely: (a) New emission sources and uses of PFCA homologues ( $C_4-C_{14}$ ) are identified. Interestingly, the short-chain PFCAs ( $C_4-C_7$ ) and their derivatives are revealed to have been manufactured in large quantities for many years before the industry started the transition to alternative substances after 2000. (b) The sources of each PFCA homologue are individually assessed for three periods (1951-2002 (pre phase-out), 2003-2015 (transition after phase-out) and 2015-2050 (prognosis)). Thus, the relative contributions of direct and indirect sources to overall emissions are identified in a homologue-specific and time-specific way. (c) Discussion of environmental release media and geographical distribution of emissions as a function of time is included with special attention given to the macroeconomic trend of manufacturing moving to Asia. (d) Information on new commercial alternatives to the long-chain PFCAs and their potential precursors in various applications (e.g. per- and polyfluoroethers as polymerization aids, fluorotelomer-based surfactants in AFFF, etc.) is compiled.

**308 Microbial Degradation of Commercial Model Fluorotelomer Monomers in Aerobic Soils** L.S. Lee, Purdue University / Dept. of Agronomy Crop, Soil & Environmental Sciences, Purdue University / Dept. of Soil, Crop & Environmental Sciences; K. Dasu, Purdue University / Department of Agronomy, Crop, Soil and Environmental Sciences, NRC Post Doc at US EPA / Office of Research and Development, National Risk Management Research Laboratory, U.S. Environmental Protection Agency / National Risk Management Research Laboratory; L.A. Royer, Exponent, Inc. / Agronomy – Crop, Soil and Environmental Sciences, Exponent Inc; J. Liu, University of Maryland Center for Environmental Science / Chesapeake Biological Laboratory, McGill University / Civil Engineering and Applied Mechanics. The global distribution of polyfluoroalkyl substances (PFASs) continues to be a concern for scientists and regulators alike. Most studies to date focus on fluorotelomer alcohols (FTOHs) and perfluoroalkyl carboxylic acids including perfluorooctanoic acid (PFOA). Probing new potential sources of PFOA is needed. We hypothesize that in aerobic soils, 8:2 fluorotelomer (FT)-based monomers will undergo hydrolytic cleavage to yield 8:2 FTOH as the primary degradation product followed by production of the suite of PFASs observed in aerobic 8:2 FTOH biotransformation. The biotransformation potential of six model 8:2 FT monomers was quantified: fluorotelomer acrylate (FTA) and fluorotelomer methacrylate (FTMA), fluorotelomer stearate ester (FTS), fluorotelomer citrate triester (TBC), an aromatic fluorotelomer diurethane (FTU) and an aliphatic fluorotelomer diurethane (HMU). FT monomers were incubated for ~1 to 7 mo in an agricultural soil or a forest soil. Abiotic controls included solvent (no soil) and autoclave-sterilized soil controls. FT compounds were applied quantitatively either dissolved in acetone or coated on talc to achieve a starting concentration of ~100 to 250 mg/kg soil. At designated sampling times, headspace samples were taken and analyzed for FTOHs, and followed by extracting soils with ethyl acetate, MTBE, or tetrahydrofuran for FT monomers and 8:2 FTOH, and then twice with 90/10 v/v acetonitrile/NaOH (200 mM). PFASs were analyzed by GC/MS or liquid chromatography tandem electrospray ionization mass spectrometry (HPLC/ESI-MS/MS). Degradation rates were affected by the non-fluorinated terminus or backbone, soil type and soil storage time. FTA degraded the fastest with half-lives of ~3 to 5 d followed by FTS (5 to 28 d) and FTMA (~15 d). For the larger FT monomers, biodegradation was slow with  $56 \pm 19$  mol% TBC remaining after 7 mo, 70 mol% FTU after 4 mo, and 73 mol% HMU after 6 mo. PFOA levels reached 1 to 10 mol% observed for the different FT-soil combinations with no apparent plateau in some studies. Storage of the forest soil around 4 °C, which is hypothesized to increase in fungal populations, greatly enhanced degradation rates of FTS. Addition of a non-fluorinated carbon source and re-aeration enhanced PFOA production, but not necessarily monomer degradation. Monomers with slower transformation rates appeared to lead to more irreversible binding of PFASs to soil, thus lower PFOA production and lower overall mass balance.



### 309 Preliminary studies on re-mobilization and metabolism of soil-bound <sup>14</sup>C-labeled 5:3 polyfluorinated acid by earthworm *Eisenia fetida*

N. Wang, DuPont, E.I. du Pont de Nemours & Company, DuPont; L.M. Sulecki, B.W. Wolstenholme, R.C. Buck, J.T. Gannon, DuPont. The 6:2 fluorotelomer alcohol [6:2 FTOH, F(CF<sub>2</sub>)<sub>6</sub>CH<sub>2</sub>CH<sub>2</sub>OH] is a major raw material to replace the 8:2 fluorotelomer alcohol (8:2 FTOH, F(CF<sub>2</sub>)<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>OH) to produce 6:2 FTOH-based products for industrial and consumer applications. Aerobic biotransformation of 6:2 FTOH in various environmental matrices (e.g., soil, activated sludge, and sediment) leads to two major classes of transformation products: perfluorinated carboxylic acids and 5:3 polyfluorinated acid [5:3 acid, F(CF<sub>2</sub>)<sub>5</sub>CH<sub>2</sub>CH<sub>2</sub>COOH]. The 5:3 acid can be further degraded in diluted activated sludge to 4:3 acid and perfluorobutanoic and perfluoropentanoic acids with a total molar yields of ~21%. In soil, however, the 5:3 acid formed during 6:2 FTOH biotransformation become tightly bound to soil organic components and is not extractable by organic solvent alone and can only be recovered by post-treatment of the organic extracts with strong base plus activated carbon. Also, only about 1 mol% of 4:3 acid is formed during 6:2 FTOH biotransformation, indicating that the soil-bound 5:3 acid was not readily available for further biodegradation. The present study investigated the bioavailability of soil-bound 5:3 acid trying to answer two questions: 1) Can earthworms remobilize the soil-bound 5:3 acid? and 2) can earthworms metabolize the soil-bound 5:3 acid to release free 5:3 acid for further biodegradation? Earth worm (*Eisenia fetida*) colonies were established in clean soil with supplementary diet. Aged soil containing soil-bound <sup>14</sup>C-labeled 5:3 acid was used for the study. The aged soil was extracted at least twice with acetonitrile to remove free 5:3 acid prior to mixing with clean soil. The earth worms were then exposed to the mixed soil for ~5 weeks with minimum diet supplement to encourage the earthworms to consume the soil. LC/MS/MS and LC/ARC (liquid chromatography/accurate radioisotope counting) were used to quantify <sup>14</sup>C-labeled 5:3 acid and potential metabolites. Detailed experimental methods and study results will be presented.

### 310 Estimating pesticide exposure and accumulation in terrestrial phase amphibians

R. Van Meter, Environmental Protection Agency / Ecosystems Assessment Branch; S.T. Purucker, Environmental Protection Agency. Dermal exposure presents a potentially significant but understudied route for pesticide uptake in terrestrial amphibians. Although some toxicity data exists for amphibians, the focus has largely centered on early aquatic life stages. Beyond providing protection and minimizing desiccation, amphibian skin has unique characteristics for gas and water exchange that may make this group very susceptible to contaminants relative to amniotes. Hydrophilic pesticides are very likely to be taken up through amphibian skin and this includes uptake from contaminated soil and foliage by terrestrial amphibians at any life stage. Our study was designed to measure dermal uptake or total body burden of pesticides of varying hydrophobicity through terrestrial exposure to juvenile frogs. In doing so, our goal is to begin to expand on the limited data currently available on toxicity of pesticides through dermal exposure in terrestrial amphibians. During spring and early summer 2012, we collected southern leopard frogs (*Rana sphenoccephala*), American toads (*Bufo americanus*), Fowler's toads (*Bufo fowleri*), gray treefrogs (*Hyla versicolor*) and tiger salamanders (*Ambystoma tigrinum*) from ponds in the University of Georgia's Whitehall Forest protected area. All amphibians were reared on-site at the US EPA in Athens, GA through 60-days post-metamorphosis. Our experimental design was a 4x2 full-factorial design with 5 replicates of each treatment as follows: 3 pesticides plus a control and 2 application treatments (pesticide mixed directly into soil or applied via overspray). Juveniles of each species were placed in glass aquariums lined with 2 centimeters of soil and exposed to pesticides through the application treatments detailed above for 12 hours. At the termination of the study, frogs were euthanized and stored in a -20 °C freezer until whole body tissue analysis was performed on a GC/MS. Tissue analysis is on-going, but preliminary results indicate that terrestrial phase amphibians do readily absorb hydrophilic pesticides from the soil environment. The dermis represents an important pathway for contaminant accumulation in terrestrial amphibians and as such, we plan to use this data to evaluate a proposed EPA dermal uptake screening tool.

### 311 Constructed and natural wetlands as sources of current-use pesticides to amphibians

K.L. Smalling, W. Battaglin, US Geological Survey; R. Reeves, Iowa State University; E. Muths, M. Vandever, M.L. Hladik, A. Gallant, W. Sadinski, D. Kolpin, US Geological Survey. Degradation and

loss of habitat due to agricultural practices are among the primary reasons amphibian populations have declined worldwide. Since the mid-1800s, agriculture has transformed the landscape of Iowa's Des Moines Lobe, the southernmost portion of the Prairie Pothole Region, from grasslands and wetlands to one of the most intensively farmed areas in the world, with only small patches of native vegetation and relatively few wetlands remaining. Despite such dramatic landscape changes, populations of native amphibians continue to persist where combinations of uplands and wetlands enable sufficient survival, reproduction, and recruitment, including areas with constructed wetlands and agricultural ditches. However, the stability of these populations and their long-term persistence in the face of multiple potential stressors is uncertain, which underscores the need for assessing the risks these stressors pose to amphibian fitness. The principal potential threats to amphibian species of the Des Moines Lobe are further habitat loss and degradation due to agricultural practices, climate change, and disease. For this reason pesticides are receiving increasing attention as a potential cause of declines, acting singly or in combination with other stressors such as disease. The current study was designed to focus on the potential exposure and accumulation of agricultural pesticides in native leopard frogs (*Lithobates pipiens*) collected from constructed and natural wetlands located in the Des Moines Lobe in Iowa. Surface water, sediment and frog livers collected throughout the spring and summer of 2012 will be analyzed for over 90 current-use pesticides and pesticide degradates including 34 fungicides using gas chromatography mass spectrometry. Preliminary results from April (prior to major crop planting efforts) indicate that a variety of pesticides were detected in water samples collected from both the constructed and natural wetlands including neonicotinoid insecticides, the herbicides, atrazine and metolachlor, and several fungicides. Pesticide concentrations in surface water from these wetlands ranged from 1 to 200 ng/L with a median of 58 ng/L. The results of the study will provide critical information on the potential for amphibians to be exposed to and take up these compounds and will aid in the assessment of subsequent risks to their fitness and long-term population persistence.

### 312 Acute toxicity of the fungicide chlorothalonil to two amphibians, *Xenopus laevis* and *Spea multiplicata*

S. Yu, Texas Tech University / Department of Environmental Toxicology; M. Wages, Texas Tech University/TIEHH / Department of Environmental Toxicology; G.P. Cobb, Baylor University / Department of Environmental Science; J.D. Maul, Texas Tech University / The institute of environmental and human health, Texas Tech University/TIEHH / Department of Environmental Toxicol. As a broad-spectrum fungicide, chlorothalonil has been widely used in agriculture to protect crops and in urban environments for lawn and garden applications. Despite being one of the most heavily used fungicides, little is known about the effects of chlorothalonil on amphibians. The objective of this study was to determine the acute toxicity of chlorothalonil to African clawed frog *Xenopus laevis* embryos (NF stage 8-11) and tadpoles (stage 45) and New Mexico spadefoot toad *Spea multiplicata* tadpoles (Gosner stage 25). We performed 96-h static toxicity tests and exposed embryos and tadpoles to control and five concentrations of chlorothalonil. At the end of the exposure period tadpoles were euthanized, examined for abnormalities, and morphological measurements were taken. For *X. laevis*, the 96-h LC50s (95% CI) were 42.1 µg/L (39.7-44.9) for embryos and 11.7 µg/L (10.4-12.7) for larvae. The results were confirmed in a second verification experiment with LC50s of 29.3 µg/L (27.1-31.8) and 17.5 µg/L (16.3-19.0) for embryos and larvae, respectively. *Spea multiplicata* tadpoles were more sensitive to chlorothalonil with an LC50 of 8.6 µg/L (7.8-9.5). We compared the LC50s of *X. laevis* and *S. multiplicata* tadpoles to those reported in invertebrates and fish, and amphibians appear to be more sensitive than invertebrates and fish. Chlorothalonil induced gut malformations in *X. laevis* embryos and high concentrations produced significantly smaller tadpoles compared to control ( $P < 0.05$ ). However, the size of *S. multiplicata* tadpoles was not significantly different among treatments. Tail shortening was observed in both *X. laevis* and *S. multiplicata* tadpoles exposed to chlorothalonil; chlorothalonil treated groups had significantly lower tail length to total length ratios ( $P < 0.05$ ). The mechanism of tail shortening induced by chlorothalonil is unclear but alteration in the expression of genes involved in tail resorption during metamorphosis may be an explanation. Although chlorothalonil concentrations in the environment were generally low (< 0.1 µg/L), high concentrations (50-130 µg/L) have been reported in run-off after application by rainfall which could cause high mortality and malformations. Our results suggest that chlorothalonil is highly toxic to amphibians, and can cause tail

degeneration in larvae which may have a significant impact on predator avoidance, the ability to acquire food, and metamorphosis.

**313 An Examination of the Maternal and Paternal Contributions to Responses of Embryonic and Larval Southern Toads (*Anaxyrus terrestris*) to Copper** R.W. Flynn, University of Georgia / Savannah River Ecology Laboratory, Savannah River Ecology Laboratory; D. Scott, S. Lance, University of Georgia / Savannah River Ecology Laboratory. Chemical contaminants, including metals, are common features in many aquatic habitats. Chemical contamination is one of the anthropogenic stressors implicated in global amphibian declines. Often the level of any given contaminant is high enough to reduce population size but not to extirpate the population through direct effects. If heritable genetic differences are associated with differential survival of individuals within a population, selection for tolerance to the given stressor along with any linked traits may occur. Though variation in response to contaminants is well documented, the basis for that variation is rarely investigated and the potential for adaptation is largely unknown. In fact, when geographic variation in tolerance corresponds with current presence/absence of a stressor it has been taken as evidence for local adaptation. These studies are correlative, but continue to demonstrate that variation in tolerance to stressors is common in amphibians. In southern toads, *Anaxyrus terrestris*, we have found substantial within and among population variation in tolerance to copper (Cu). To gain a better understanding for whether that variation has an underlying genetic basis we artificially fertilized eggs of five female southern toads with sperm from four male toads from the same population. This resulted in groups of full siblings and paternal and maternal half siblings. Half siblings allow for analysis of genetic and phenotypic contributions of mothers and fathers individually, as well as the interaction of the two. This is essential to quantitatively determine whether there is heritable genetic variation for tolerance to stressors and other quantifiable traits that could result in adaptation. We exposed embryos to a range of Cu treatments so that maternal, paternal, and maternal x paternal contributions to the response of embryos and larvae could be quantified. Embryonic survivorship was significantly affected by Cu treatment and parentage, with a significant maternity x paternity interaction. The response to Cu was significantly different among families and was affected more by paternity than maternity. In a subsequent trial we chronically exposed larvae for three weeks. We took photographs of each tadpole weekly to examine the effects of paternity, maternity, Cu, and interaction of the three on growth rate and other sublethal endpoints. Upon completion of the trial we collected all tadpoles for gene expression and SNP analysis.

**314 Individual and population level differences in response to chronic copper exposure in southern toads, *Anaxyrus terrestris*** S. Lance, University of Georgia / Savannah River Ecology Laboratory; R.W. Flynn, University of Georgia / Savannah River Ecology Laboratory, Savannah River Ecology Laboratory; K. Jones, University of Colorado, Denver / Biochemistry and Molecular Genetics; M. Erickson, Georgia Southern University / Biology; T. Tuberville, G. Mills, D. Scott, University of Georgia / Savannah River Ecology Laboratory. Environmental contaminants are one stressor implicated in the global decline of amphibian populations. There have been numerous studies examining the lethal or sub-lethal effects of contaminants on amphibians. However, we know relatively little about among individual and among population variation in response to exposure and the potential for local adaptation and population persistence. Understanding what makes some individuals and populations more resilient to anthropogenic stressors is important for conservation biology and is lacking in most ecotoxicological studies. One first step is to further examine how much standing variation in tolerance exists within and among populations prior to exposure to a specific stressor. Metals resist degradation in the environment and thus are likely to act as a chronic stressor that has the potential to expose multiple generations. We examined the effects of Cu exposure on the southern toad, *Anaxyrus terrestris*, a widespread species for which no data on Cu exposure are available. Our objectives were to: 1) examine the effects of acute and chronic Cu exposure, 2) quantify lethal and sublethal effects, and 3) assess variation in tolerance to Cu within and among populations with and without prior Cu exposure. Overall Cu significantly reduces survival through the egg and larval stages. We examined eggs from multiple clutches and source populations and both factors are significant sources of variation in survival. Clutches from populations with prior Cu exposure had the lowest survivorship. Among several source populations with no prior Cu exposure there was still significant variation in response to Cu. Depending upon the source

population survival to the free-swimming stage was significantly reduced at concentrations as low as 10 µg/L and no larvae reached metamorphosis at concentrations above 15 µg/L. Neither time to- or size at-metamorphosis were significantly affected by Cu treatment. In a separate study, to better understand the effects of Cu we used RNASeq to examine gene expression patterns in developing toads exposed to Cu. We compared expression in early development of eggs from 24 to 54 hours post treatment. At 55 hours nearly 200 genes are differentially expressed between control and treatment individuals. We discuss our findings and relate them to potential impacts on population level processes.

**315 Improving reptile risk assessment: relative importance of diet and dermal contaminant exposure in the Western fence lizard (*Sceloporus occidentalis*)** S.M. Weir, The institute of environmental and human health / The institute of environmental and human health, The institute of environmental and human health / Environmental Toxicology; L.G. Talent, Oklahoma State University / Department of Natural Resource Ecology and Management; C.J. Salice, Texas Tech University / Environmental Toxicology, Texas Tech University / TIEHH, Texas Tech University / Department of Environmental Toxicology. Reptile ecological risk assessment is currently limited by a lack of toxicity data for reptiles, and little knowledge of contaminant exposure. Recent advances in the use of the Western fence lizard (*Sceloporus occidentalis*) as a model organism creates opportunities for filling important data gaps. Interest has been growing for including dermal exposure in ecological risk assessments for birds. Because many reptiles spend a large proportion of time in contact with substrate, there is potential for dermal exposure to be important for reptiles. The purpose of this research was to provide insight into the relative importance of diet and dermal contaminant exposure in reptiles. Fence lizards were exposed to phthalate esters by either oral gavage or dermal application. Phthalates were chosen as model chemicals because lipophilicity changes with the length of the side chain allowing relationships between dermal exposure and lipophilicity to be estimated in the absence of overt toxicity. We used di-methyl phthalate (DMP, logKow = 1.6), di-iso-butyl phthalate (DIBP, logKow = 4.0), and di-n-octyl phthalate (DNOP, logKow = 8.0). Lizards from both exposure routes received the same dose (10 µL) of each phthalate. Following 24 or 48 hours, lizards were sacrificed and organs removed for chemical analysis. Dermal exposure was highest for phthalates with greater lipophilicity, but was greatest for DIBP at both 24 and 48 hours. Generally, DMP had lower concentrations for both oral and dermal exposure routes, but had many more detections in oral treatments than dermal. DIBP and DNOP appeared to be significantly absorbed by both dermal and oral exposure. Dietary exposure resulted in significantly greater liver and fat residues for both DIBP and DNOP than dermal exposure. Dermal skin samples had very high concentrations of phthalates indicating that only a fraction of the phthalates applied had crossed the dermal barrier. Realistic assessments of contaminant exposure in reptiles should consider dermal exposure routes, especially because reptiles feed infrequently and have relatively low oral exposure. Future research will repeat this experiment under more realistic dosing scenarios (i.e. contaminated soil and dosed food) followed by field truthing of laboratory results in terrestrial enclosures. Results from this and subsequent empirical research will be used to inform, populate and verify dermal exposure models for use in ecological risk assessments.

**316 AROD Optimization in Chorioallantoic Membranes (CAMs) of Kemp's Ridley (*Lepidochelys kempii*) sea turtles** R.J. Pezdek, Texas Tech University / Institute of Environmental and Human Health, Texas Tech University; D.J. Shaver, US National Park Service / Padre Island National Seashore; M.J. Hooper, R.L. Tillitt, USGS / Columbia Environmental Research Center; J.E. Yacabucci, Texas Tech University / Institute of Environmental and Human Health; J.S. Walker, S.C. Ertolacci, US National Park Service / Padre Island National Seashore; C.J. Godard-Codding, The Institute of Environmental and Human Health/ Texas Tech University / Assistant Professor, Texas Tech University/The Institute of Environmental and Human Health / Assistant Professor, Texas Tech University / Institute of Environmental and Human Health. Reptiles are present worldwide and play a key ecological role in various environments. Previous research on declining reptilian species is minimal and leaves many questions unanswered, such as the effects of environmental contaminants. Sea turtles are a key reptilian species in the marine environment and understanding impacts on their health is crucial to conserving currently declining sea turtle populations. With only seven sea turtles species in the world, all of which

are threatened or endangered, traditional *in vivo* toxicology experiments on this species are prohibited. The chorioallantoic membrane (CAM) is a highly vascularized egg membrane involved in the respiration and waste storage for the developing embryo. The CAM's physiological role has often been compared to that of the liver, and previous studies have shown that contaminant burdens found in the CAM correlate well with body burdens in the embryo. The CAM is attached to the outer shell and surrounds the embryo until hatching, when it remains behind in the eggshell. This makes the CAM an excellent tissue for assessing biomarkers of environmental contaminant exposure and effects for non-invasive wildlife research and a novel research tissue for endangered sea turtles. We collected Kemp's ridley sea turtle CAMs during the 2010 nesting/hatching season from Padre Island National Seashore in Corpus Christi, TX (permit #'s TE840727-3 and SPR-0190-122). Here, we report on the optimization of cytochrome P450 catalytic analyses in CAM for use in alkoxyresorufin O-dealkylation (AROD) assays. We investigated (1) four different homogenization steps (whole tissue homogenization, 300 xg, S9, and microsomal preparation), (2) protein loss between homogenization levels, (3) sonication versus traditional homogenization methods, (4) mean weight of CAM tissue, and (5) mean amount of protein found in CAMs. Our preliminary results indicate that the S9 purification level appears best suited for analyses in CAMs weighing 0.15g or more by providing enough protein for the analyses of the four primary AROD assays: 7-ethoxyresorufin O-dealkylation (EROD), 7-methoxyresorufin O-dealkylation (MROD), 7-pentoxoresorufin O-dealkylation (PROD), and 7-benzoyloxyresorufin O-dealkylation (BROD). Further sample analysis will help elucidate which AROD(s) may be best suited for the detection of cytochrome P450 catalytic activity in sea turtles.

**317 In vitro Toxicity of Perfluorinated Compounds in Loggerhead Sea Turtle (*Caretta caretta*) Primary Skin Cell Cultures** S.J. Webb, Texas Tech University / The institute of environmental and human health, The institute of environmental and human health; B.M. Higgins, NOAA/NMFS; C.A. Godard-Coding, Texas Tech University / The institute of environmental and human health. Perfluorinated compounds (PFCs) are globally distributed contaminants which have been measured in both human and wildlife blood and serum. Sources include polymerization aids, stain repellents, surfactants, and medical devices. These compounds have been shown to cause a variety of adverse health effects in mammals, including the development of tumors in the liver and thyroid, hepatotoxicity, developmental delays, interruption in lipid metabolism and adipogenesis leading to decreased body weight, and a significant increase in both internal and external morphological abnormalities. There is currently no PFC toxicity data available in any reptile species. PFCs are found in loggerhead sea turtle tissues at levels which can cause significant toxicity in mammals. In loggerhead sea turtles perfluorooctanoic acid (PFOA) and perfluorooctanesulfonate (PFOS) are the dominant PFC compounds found in serum. Here, we report on the use of cytotoxicity assays to assess adverse effects of PFOA in sea turtles. Loggerhead sea turtle primary skin cell cultures which were previously established and characterized in our lab were used for testing. Because PFCs are known to affect mitochondrial activity and lipid production in mammalian toxicity studies, (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and lactate dehydrogenase (LDH) were selected as initial toxicity assays. Initially, a variety of test concentrations (0.05  $\mu$ M, 0.5  $\mu$ M, 5.0  $\mu$ M, 50  $\mu$ M, 500  $\mu$ M) were used, which encompass chemical concentrations found in the environment, in turtle serum, and in prey animals. Significant toxicity occurs at the highest dose (500  $\mu$ M) with cells from n=6 turtles for MTT and n=5 turtles for LDH, a large sample size when working with endangered species. Following initial testing, dose range was narrowed between the highest dose (500  $\mu$ M) and the highest dose showing no significant toxicity (50  $\mu$ M). Both MTT and LDH assays were performed on this second dose range (50  $\mu$ M, 140  $\mu$ M, 230  $\mu$ M, 320  $\mu$ M, 410  $\mu$ M, 500  $\mu$ M). Significant toxicity occurs at 410  $\mu$ M and 500  $\mu$ M with cells in initial testing.

**318 Effect of Sediment Physicochemical Properties on the Bioavailability of Sediment-Associated Uranium to a Model Benthic Organism, *Chironomus dilutus*** S.E. Crawford, L. Doig, K. Liber, University of Saskatchewan / Toxicology Centre. The importance of metal bioavailability in aquatic ecosystems has been well established. However, our understanding of the factors and mechanisms controlling the bioavailability of some metals is still incomplete. In particular, there is limited information regarding bioavailability and toxicity of uranium (U) in freshwater sediment. Mineral coatings on particle surfaces have been shown to adsorb metals. Given that

dissolved U is believed to be the primary bioavailable fraction of U, it is hypothesized that adsorption and interaction of U with various mineral coatings will influence the bioavailability of U in sediment. As such, the goal of this research is to investigate the effects of specific sediment physicochemical properties, mainly clays and organic matter, on the bioavailability of U to a model freshwater benthic organism, *Chironomus dilutus*. Differences in binding capacities and hence changes in bioavailability were examined in a series of 10-d sediment bioaccumulation/toxicity tests, exposing 8-d old *C. dilutus* larvae to different treatments of U-spiked sediment. Test endpoints and measurements included *C. dilutus* survival and growth, and U concentrations in whole organisms, whole-sediment and water (both overlying water and pore water). Studies were conducted with 0 (control), 50 and 200 mg U/kg dry weight (d.w.) spiked into formulated sediments and aged for 20 d. Composition of sediments varied according to the characteristic being examined, but included silica sand as the foundation of the mixtures, along with one of three different types of clay (kaolin, montmorillonite, and illite) added at 0 to 60% of the sediment d.w., or peat moss added as a source of organic matter at 0, 5, or 15% of sediment d.w. Bioaccumulation of U in *C. dilutus* larvae ranged from 10 to 1195 mg U/kg d.w. when exposed to 50 mg U/kg d.w. in sediments containing 0 to 60% clay (d.w.), respectively. Similarly, higher organic matter content was associated with lower amounts of U bioaccumulation in *C. dilutus* larvae, indicating a reduction in U bioavailability. It was also found that U bioavailability differed among the three types of clay minerals examined, with bioavailability decreasing with montmorillonite > illite  $\approx$  kaolin clay. Results from this research will guide future studies with more complex sediment, while also informing risk assessments of U contaminated sites and possibly the development of site-specific sediment quality guidelines for U.

**319 Metal Effects on Benthic Invertebrates in Off-Site Ponds near the Deloro Mine Site (Ontario, Canada)** L.E. Brown, ENVIRON International Corporation; P.C. Fuchsmann, ENVIRON International Corporation, ENVIRON International Corp.; M. Henning, ENVIRON International Corporation. Historical mining and smelting operations at the Deloro Mine Site resulted in elevated concentrations of arsenic, cobalt, copper and nickel in sediments in Young's Creek and associated beaver ponds. We investigated risks to benthic invertebrates in these off-site sediments through a novel application of the sediment quality triad (SQT) approach. In addition to the typical SQT elements – sediment toxicity testing, benthic community taxonomic characterization, and sediment chemistry – bioavailable exposures were evaluated by analyzing metals in amphipod tissues and copper in water overlying toxicity tests. Toxicity tests results for three species tended to over-predict effects compared to the benthic community data; overlying water copper data suggest possible laboratory artifacts (e.g., low dilution compared to field conditions). Consequently, site-specific toxicity reference values (TRVs) were derived from the benthic community data set. Taxa richness, abundance, Shannon diversity, and Hilsenhoff Biotic Index were the most useful metrics for differentiating benthic community quality across sampling locations. The influence of each metal relative to observed effects varied across sampling locations. Therefore, overall benthic quality was ranked, and apparent effect thresholds were identified based on tissue or overlying water exposures. Organic carbon normalization of sediment metal concentrations enabled us to translate the apparent effect thresholds to sediment TRVs, while maintaining accurate predictions of effects. The resulting site-specific TRVs are considerably higher than default sediment screening values, which in turn has large impact on estimated costs to remediate the sediment.

**320 Assessing risk in a contaminated river: links between abiotic and biotic factors as predictors of tissue metal concentrations in a stream biomonitor** M. Hornberger, D. Cain, U.S. Geological Survey / Water Resources Division; M. Croteau, U.S. Geological Survey. Assessments of stream health using dissolved and sediment metal concentrations provide a basis for determining possible adverse effects to aquatic organisms. Tissue residue studies using resident species provide biological relevance because organisms integrate metal from their surrounding environment, thus representing bioavailable metal. Biodynamic models can identify key the physiological processes and exposure routes linking abiotic measures of metal exposure and metal concentrations in resident organisms. This approach is being integrated with long-term monitoring field study to evaluate remediation of mining wastes at an USEPA Superfund site. Field work, laboratory experiments and biodynamic modeling can help identify the mechanisms controlling bioaccumulation, allowing us to 1) quantify the routes of



exposure to a larval insect that is being used to monitor bioavailable metals; 2) identify the time period of exposure represented by tissue concentrations in the biomonitor; and 3) determine exposure scenarios necessary to reach reference values. We present an integrated approach to assessing the success of remediation efforts on a metal-impacted river using the common biomonitor *Hydropsyche* (O: Trichoptera). Physiological processes (uptake and loss), and exposure routes (dissolved and diet) were quantified using an enriched stable isotope tracer ( $^{65}\text{Cu}$ ) and biodynamic modeling. Site-specific geochemical field data were incorporated into a biodynamic model, allowing us to validate the model parameters and predict copper bioaccumulation in *Hydropsyche*. Our findings suggest that dietary Cu influx is ~3-fold higher than dissolved influx and that consideration of dissolved only exposure under-predicts *Hydropsyche* Cu concentrations by ~70%. *Hydropsyche* also has fast uptake and loss rates, which has important implications for monitoring programs. This species can reach steady state in ~30 days, but eliminates 17-25% of its Cu body burden per day if exposure is removed. Fluctuations in river discharge also influence exposure and is linked to the physiological and behavioral mechanisms controlling Cu uptake in *Hydropsyche*. Understanding how these abiotic and biotic factors are coupled will allow us to predict how tissue concentrations will change under different exposure scenarios.

**321 Evaluation of Statistical Bioaccumulation Models for Prediction of Tissue and Sediment Concentrations** N. Judd, J. Toll, Windward Environmental LLC; L. Tear, Windward Environmental. Biota-sediment accumulation factors (BSAFs) or biota-sediment accumulation regressions (BSARs) are statistical tools used to estimate chemical concentrations in tissue from sediment concentrations when empirical tissue data are lacking, estimate tissue concentrations under alternative future conditions (e.g., remedial actions or dredging), or estimate sediment preliminary remediation goals (PRGs) from target tissue concentrations. Scatter plots and linear (or non-linear) models offer managers the ability to explore the relationship between sediment and tissue concentrations at a site and make decisions that acknowledge the shape and uncertainty of the relationship. Data from the US Environmental Protection Agency Mid-Continent Ecology Division's BSAF database were used to explore tissue-sediment relationships. Of the 262 datasets for benthic invertebrates with sufficient data for evaluation, many of the sediment-tissue relationships were negative and about two-thirds of the relationships were very poor and did not meet minimum screening criteria (i.e.,  $r^2 > 0.3$ ,  $p\text{-value} < 0.05$ ). Further evaluation of three of the stronger relationships was performed. BSAFs, linear regressions, Model II regressions, example sediment PRGs, and associated confidence intervals were calculated for each example. The examples illustrate several simple but important aspects of sediment-tissue relationships that should be explored for their effects on BSAR and BSAF applications and on the statistical uncertainty of the relationships: non-zero intercept for the relationship (i.e., accounting for non-sediment sources), the nature and shape of the relationship (e.g., linear, log-linear, or non-linear), and the effect of individual sediment-tissue pairs on the relationship (e.g., the influence of a single high point). Simple models, like BSARs and BSAFs, may continue to be the best options for decision-making in many applications; however, their limitations and uncertainties should be acknowledged and quantified when possible. Some best practices for developing and applying sediment-tissue relationships are also proposed.

**322 Selenium biodynamic modeling: Can it be done? Should it?** S.D. Baker, GEI Consultants, Inc. / Ecotoxicologist/Wildlife Biologist; S.P. Canton, GEI Consultants, Inc. / Ecological Division; E. Smith, C. Claytor, GEI Consultants, Inc.. The U.S. EPA is currently revising the national ambient water quality criteria (AWQC) for selenium (Se). It is expected that the new criteria will include a component based on Se concentrations in fish tissues. Implementation of tissue-based standards would require the collection of tissues from wild populations of fish for water quality criteria attainment assessments, which would be an added expense and potentially difficult for both regulatory agencies and the regulated community (i.e., tissue samples would be required in addition to typical surface water samples). Thus, it is expected the U.S. EPA will include in its criteria document a recommended methodology for back-calculating a protective water column concentration from the tissue-based standard, allowing for monitoring of Se in surface waters instead of tissues. Biodynamic modeling has been proposed (e.g., Presser and Luoma 2010) as a potential method for estimating allowable site-specific surface water Se concentrations that will maintain the protection level intended by a given tissue-based standard. While biodynamic

modeling incorporates important site-specific characteristics, which allows for calculation of relevant water-based standards for a given site, the inherent range of values for model parameters can result in widely variable results. In addition, this data variability complicates model calibration and validation (i.e., using site-specific model parameters to predict known site fish tissue concentrations). In this analysis, we used water, sediment, benthic invertebrate, and fish tissue selenium data from two study areas in Colorado to develop site-specific biodynamic models. Validation of these models was attempted using multiple mathematical approaches, including those suggested by Presser and Luoma (2010). Models developed using site-specific data could not be validated, and depending on how data were used, simple bioaccumulation factor (BAF) calculations were identical to biodynamic model results. These results raise a number of questions, including whether it is worth the time and expense for the additional data collection required to run the biodynamic model and whether it is possible to develop any type of truly predictive site-specific bioaccumulation model for Se. Such a model is necessary before any "back-calculated" water-based standards can be adopted.

**323 The Biodynamic Model: a means to improve sediment bioaccumulation protocols and a potential regulatory tool?** H. Selck, Roskilde University / Department of Environmental, Social and Spatial Change, Roskilde University / Dept. Environmental, Social and Spatial Change; S.N. Luoma, University of California Davis / John Muir Institute of the Environment, U.S. Geological Survey, University of California, Davis; M. Croteau, U.S. Geological Survey. To perform environmental risk assessment and to develop robust predictive bioaccumulation models that can be used in a regulatory context, it is vital that we understand and are able to determine the transfer of contaminants from the abiotic (water, sediment, diet) to the biotic compartment. From a regulatory perspective, accumulation from the sediment compartment is important because sediments act as accumulation sites for metals and hydrophobic compounds, and because increasing evidence shows that particle ingestion is the main route of contaminant uptake in deposit-feeders. Benthic communities are therefore likely to play an important role in the remobilization of sediment-associated contaminants to higher trophic levels (i.e., food web transfer). The standardized test guideline, OECD 315, was developed to determine sediment bioaccumulation in sediment-dwelling benthic organisms. Some of the challenges associated with this test includes the relatively long duration (28d uptake, 10d elimination), the use of artificial sediment, a relatively large amount of sediment per test as well as the use of the oligochaete worm, *Lumbriculus variegatus*, as a representative for deposit-feeding organisms. Here we propose and discuss the biodynamic model as a regulatory tool and as a means to develop an alternative standardized test protocol to determine bioaccumulation kinetics from both water and sediment/diet. One of the strengths of the biodynamic model is that it takes into account all uptake routes and allows capturing the biologically driven processes that govern bioaccumulation among species both for metals and organic compounds. In addition, relatively simple short-term experiments allow determination of the required unidirectional fluxes: influx from water and diet as well as efflux (loss and growth). Each biodynamic parameter is measured without interference from simultaneous processes and is used to determine accumulated whole body concentrations (i.e., balance between these opposing fluxes). An important capacity of the model, in a regulatory context, is the potential to include site-specific concentrations, geochemical conditions and species-specific comparable rate constants to predict contaminant bioaccumulation.

**324 Assessment of the Relative Contribution of Ongoing Water Column Versus Sediment Sources of PCBs to Dungeness Crabs From Victoria Harbour, BC** W.R. Hovel, Weston Solutions, Inc., Anchor QEA; E.M. Lamoureux, Anchor QEA, LLC, Anchor QEA; M. Larsen, Anchor QEA; G. Watson, Transport Canada / Environmental Officer; E. Shankie, Public Works Government Services Canada; P. Allard, Azimuth Consulting Group; G. Mann, Azimuth Consulting Group, Inc.. A Dungeness crab hepatopancreas consumption advisory is currently in effect for Victoria Harbour, British Columbia as a consequence of elevated concentrations PCBs, dioxins, and furans. In addition to localized areas of sediment contamination, elevated concentrations of PCBs have been measured in stormwater; however, prior to this study, the relative contribution of these contaminants from ongoing water column sources (i.e., stormwater) versus localized sediment sources to the Victoria Harbour food web was unknown. The current study objective was to evaluate the relative contribution of water column

PCB sources to the crabs through data analysis and bioaccumulation modeling to develop long-term management alternatives for Victoria Harbour. A targeted sampling program was conducted to assess concentrations of PCBs and other site-specific physical and chemical parameters in water, sediment, and representative organisms from Victoria Harbour. Delta  $^{15}\text{N}$  and  $^{13}\text{C}$  stable isotope data collected as part of an earlier study were used to characterize the food web and diet of key organisms. The Arnot and Gobas (2004) AQUAWEB v1.2 bioaccumulation model was used to evaluate the relative contribution of sediment and water PCBs to the Harbour food web. Modeling results provided a reasonable fit to empirical data for the PCB compounds evaluated: the whole Harbour model demonstrated more accuracy in predicting crab PCB concentrations than subarea models and average model bias for crab in the whole Harbour was approximately 1.25, with the 95% LCL and UCL within a factor of 2. The subarea PCB models demonstrated reasonable prediction of perch PCB concentrations. Modeling of paired sediment-clam data indicated that the model was accurately predicting PCB concentrations for clams. Sensitivity analyses demonstrated the importance of sediment and water PCB concentrations, and diet of perch and crab on model outputs. The largest sources of uncertainty to the model included limitations in data used for model inputs and high variability of sediment exposure concentrations. Results suggested that risk management alternatives for the Harbour should include the consideration of ongoing water column sources of PCBs, in addition to localized sediments sources, to PCB tissue concentrations.

**325 Towards ecologically relevant sediment quality criteria** F.A. Gobas, Simon Fraser University / School of Resource and Environmental Management (Faculty of Environment), Simon Fraser University / School of Resource & Environmental Management, Faculty of Environment, Simon Fraser University; J. Arblaster, Simon Fraser University / Resource & Environmental Management; P. Wing Ho Luk, Simon Fraser University / Biological Sciences; J.A. Arnot, ARC Arnot Research & Consulting Inc., University of Toronto Scarborough / Department of Physical and Environmental Sciences. Sediment quality criteria for contaminants are widely used to conduct risk assessments, to develop goals for remediation and to set permit levels for ocean disposal of contaminated sediments. In most cases, the sediment quality guidelines are based on toxicity information for benthic invertebrates and aim to protect benthic invertebrate populations. The sediment quality criteria are in most cases not suitable to protect other organisms such as fish, crabs, marine mammals, birds and human populations who consume various organisms through fishing, crabbing and other forms of hunting. This study reports on the development and application of quantitative modeling and empirical approaches to describe the relationship between contaminant concentrations in sediments, water and biota of complex food-webs for the purpose of risk assessment and the development of ecologically and human health based sediment quality criteria for contaminants that aim to protect wildlife species as well as human populations who consume wildlife. The application of the methodologies for the development of sediment quality guidelines and disposal permits for PCBs and PBDEs in marine habitats of killer whales, Steller sea lions, salmon and human consumers of wildlife is illustrated.

**326 Oil spills from pipelines – why we should be concerned?** P.V. Hodson, Queen's University / School of Environmental Studies; L.L. Williams, U.S. Fish & Wildlife Service. The traditional image of an oil spill involves sinking ships or oil rigs, crashing waves, sticky shorelines, and wildlife destruction in marine ecosystems, as exemplified by the Exxon Valdez and Deepwater Horizon incidents. However, major oil spills at sea account for only a small percentage of the total spilled annually; more than 50% is spilled on land because of a high frequency of small accidents due to transportation, production, refining, and use of oil and oil products. Pipelines may be the safest way to transport large volumes of oil, but the extensive network of pipelines, many of them aging, results in frequent spills that have the potential to cause extensive damage, as exemplified by spills to the Kalamazoo River, MI (2010) and the Pine River, BC (2005). The volumes of spilled oil often exceed 1,000,000 L, but are generally smaller than in marine ecosystems. Nevertheless, the consequences can be far more serious and long-lasting because of the high ratio of river banks to water mass, lower dilution ratios, the propensity of oil and diluted bitumen to sink in fresh water, the extent to which sediments and riparian lands are contaminated, the lack of appropriate clean-up methods, and habitat destruction caused by clean-up. We explore the challenges of exposure, hazard, and risk assessment

for freshwater and terrestrial receptors exposed to pipeline spills and identify critical research needs to improve response capabilities.

**327 Forensic Identification And Quantification of Oil Sands-Based Diluted Bitumen Released Into a Complex River Environment – The Kalamazoo River Spill** G. Douglas, NewFields Environmental Forensics / Environmental Monitoring & Analysis; B. Hollebhone, Environment Canada / Emergencies Science & Technology. Accurate identification and quantification of spilled petroleum in marine and aquatic sediments is a critical component of the Natural Resource Damage Assessment process. The Kalamazoo Oil Spill represents a unique opportunity to investigate the transport and fate of low API gravity Canadian tar sands bitumen/condensate mixture within a river environment that has a substantial background hydrocarbon signature from other petroleum releases, atmospheric deposition, biogenic sources, and land runoff. Once released to the Kalamazoo River, the light condensate was rapidly lost through evaporation and the remaining oil formed small droplets or globules in the water column that settled out in the river bed sediments. Poling, a process of disturbing the sediment bottom to assess the level of sediment contamination produced light, moderate, heavy surface sheens at water temperatures greater than 60°F, a temperature below which the oil forms a semi-solid. The ability to identify and quantify this oil in the river however, was confounded by the high level of background hydrocarbons, and the remediation method of mixing the upper several feet of sediment to release the trapped oil for recovery. In this work, we document a technical approach to 1) identify the chemical signature of the spilled oil in the mixed sediment layer, and 2) quantify the amount of spilled oil in the sediment. Three types of samples were collected to perform this analysis, oil globules in the sediment core, whole sediment and sheen from the surface water. These sediment samples were analyzed by high resolution gas chromatography with mass spectrometry (GC/MS) and flame ionization detection (GC/FID) for polycyclic aromatic hydrocarbon (PAH) /sulfur heterocyclics /biomarkers and alkanes/total petroleum hydrocarbons (TPH) respectively. The chemical signatures were evaluated using a normalized mixing model relative to the source oil, oil globule, sheen and whole sediments where the sediment specific oil signature was extracted from the whole sediment signature, generating a residual hydrocarbon distribution consistent with sediment background. These results will be used to support the NRDA and to accurately delineate the extent of oil transport and degradation in the river sediments.

**328 Fate of the Enbridge Line 6B oil in the Kalamazoo River** M. Boufadel, New Jersey Institute of Technology / Civil and Environmental Engineering; K. Lee, Department of Fisheries and Oceans; R. Zelt, F. Fitzpatrick, I. Cozarella, USGS. The rupturing of the Enbridge Line 6B pipeline at Marshall, Michigan resulted in the spillage of a million gallon of heavy crude. The spill contaminated approximately 40 miles of the Kalamazoo River reaching to Morrow Lake. As of May 2012, efforts were still ongoing to restore the river to its prior recreational usage. The binding of heavy bitumen oil with fines in the Kalamazoo River made this spill highly challenging in terms of the transport and fate of oil. We provide a brief overview of the spill and we focus on the fate of oil buried in the streambed, which is affected by the environmental conditions and local hydraulics. We report measurements of environmental conditions and the hydraulics at select locations in the streambed, both are affected by groundwater flow (hyporheic flow) and stream hydrodynamics.

**329 Immediate Assessment for Impacts to Natural Resources During a Pipeline Oil Spill into the Kalamazoo River** S. Millsap, U.S. Fish & Wildlife Service / US EPA LLRS; L.L. Williams, J. Haas, U.S. Fish & Wildlife Service; S. Hanshew, J. Wesley, Michigan Department of Natural Resources; W. Taft, M. Walterhouse, Michigan Department of Environmental Quality; J. Winter, NOAA / Office of Response and Restoration; R.T. Williamson, Match-E-Be-Nash-She-Wish Tribe of Pottawatomie; D. Beltman, A. Ebbets, K. Ritter, Stratus Consulting; D.E. Tillitt, D.M. Papoulias, U.S. Geological Survey / Columbia Environmental Research Center, USGS / Columbia Environmental Research Center; D. Nicks, USGS; P. Badra, Michigan State University. When a pipeline ruptured near Marshall, Michigan, in July of 2010 and discharged tar sand crude oil into a wetland, through Talmadge Creek, and into the Kalamazoo River, federal, state, and tribal resource agencies began almost immediately to assess impacts to natural resources from the spill. Impacts from this spill extended well into the floodplain because the river was flooded at the time of the spill and included

sediments because a significant amount of this oil sank. Federal and state biologists worked with the owners of the pipeline and their contractors to survey for and collect oiled wildlife, and then rehabilitate and release them whenever possible. Released birds were banded and turtles were PIT tagged to monitor survival and dispersal. State biologists led fish kill investigations and monitored immediate water quality. For benthic invertebrate community assessments and fisheries surveys, they were able to implement standard protocols at locations that allowed data to be compared with previous years and reference locations. Trustees for Natural Resource Damage Assessment (NRDA) worked with the pipeline owners to quickly plan and implement additional ephemeral data collection efforts. Water and sediment sampling was conducted in ecologically important locations and with an extended analyte list relative to the sampling being performed for the spill response. NRDA ephemeral data collection in the first year following the spill also included sampling of fish (including bile) and mussels, surveys for extent of oiling in floodplain habitats, monitoring vegetation for impacts, recovery and invasive species, repeated fish and invertebrate community assessments, and assessments of physical impacts of the response. Response and assessment work are continuing in 2012.

**330 Turtle Stories – Wildlife Response on the Kalamazoo River** B. Doherty, Enbridge Energy; J. Otten, Stantec, Inc.; L.L. Williams, U.S. Fish & Wildlife Service. The release of crude oil into Michigan's Kalamazoo River in July 2010 led to an unprecedented wildlife response involving the collection and care of nearly 6300 animals over a 20 month period. Of the collected animals, 5121 were turtles, representing nearly all of the estimated population within the approximately 40 miles of affected river. Issues faced in saving this many turtles included the need for multiple capture methods, addressing the effects of ongoing cleanup operations, finding effective ways of cleaning oiled turtles, treating varied medical conditions, housing and care for large numbers of turtles, overwintering turtles, finding suitable release locations, and caring for gravid turtles and hatchlings in captivity. Eight species of turtles were captured using hand nets, crab traps, and fyke nets: Spotted (*Clemmys guttata*), Blanding's (*Emys blandingii*), Eastern Box (*Terrapene carolina*), Common Musk (*Sternotherus oderatus*), Common Painted (*Chrysemys picta*), Spiny Softshell (*Apalone spinifer*), Common Snapping (*Chelydra serpentina*), and Northern Map (*Graptemys geographica*). Oiled turtles were cleaned using a variety of techniques and tools such as scrapers, scouring pads, mayonnaise, and dish soap. Medical conditions encountered included skin burns, esophageal corrosion, enlarged livers, shell lesions, and infections. Release locations for the affected animals changed over the course of the response from locations outside the spill zone but within the watershed to locations in the affected stretches of river as the cleanup became more focused. Collection late into the fall of 2010 resulted in the overwintering of 472 turtles in the wildlife center due to a concern about displacing turtles immediately prior to hibernation. A lesser number of turtles were overwintered in 2011. A number of the overwintered females were gravid, resulting in a need for special nesting tanks and incubators. In total, 158 hatchlings were born in the wildlife center and released to the wild. Overall, the survival rate for animals collected during the Kalamazoo River wildlife response was 96.3 percent, and 97.9 percent for turtles (not including the hatchlings).

**331 Bridging Oil Spill Response and Scientifically Supported Information: A Net Environmental Benefit Analysis for the Kalamazoo River Oil Spill** A.C. Bejarano, Research Planning Inc. / Research Planning Inc., Research Planning, Inc. / Department of Environmental Health Sciences Public; J. Michel, Research Planning, Inc.; F. Fitzpatrick, U.S. Geological Service; M. DeGraeve, Great Lakes Environmental Center; L.L. Williams, U.S. Fish & Wildlife Service; M. Alexander, Michigan Department of Environmental Quality; S. Hamilton, Michigan State University; J. Chapman, U.S. Environmental Protection Agency. On July 26, 2010, a pipeline rupture released ~1M gallons of diluted tar sands crude oil into Talmadge Creek and the Kalamazoo River, MI (USA). The spill happened during a flood and impacted over 30 miles of the river including channels, backwaters, wetlands, forested floodplains, and impoundments where an unknown fraction of the oil became submerged. The submerged oil recovery process in 2010-11 included a variety of methods to agitate sediments and recover the resultant sheen. Because of continued issues with submerged oil, a multidisciplinary group was formed to weigh the potential environmental effects of removing the remaining oil or leaving it in place. A Net Environmental Benefit Analysis (NEBA) was used to assess the potential ecological

consequences of oil cleanup options, including consideration of plants, invertebrates, fishes, amphibians, and reptiles in eight major riverine and wetland habitats along the Kalamazoo River. Critical to this assessment was the analysis of sediment chemistry and toxicity testing with *Chironomus dilutus* and *Hyalella azteca*, turbidity data from sediment agitation techniques extensively used for oil recovery in 2011, and a literature review of impacts of sediment agitation. The Equilibrium Sediment Benchmark Toxic Unit approach was used to assess risks from PAH mixtures to benthic organisms. Of the 20 sites chosen for toxicity testing, two heavily oiled and one lightly oiled site posed adverse risks to benthos. However, acute sediment toxicity results did not show significant reductions in survival, growth or biomass relative to a field reference. Based on the weight of evidence, residual oil in the most heavily oiled areas may pose some risks to benthic receptors. Comparison of turbidity data collected during sediment agitation and turbidity levels associated with ecological effects in fish indicated that increased turbidity from agitation may pose moderate (sublethal) effects to juvenile and adult fish species. Post-spill turbidity does not appear to exceed the historical range of turbidity values in the river. Sediment agitation, especially the direct physical impacts, potentially results in mortality of benthic invertebrates, eggs and larvae of bivalves and fish, and destruction of aquatic macrophytes. Overall, the NEBA indicated that, with the exception of certain sites along the river, natural attenuation with monitoring and sheen collection was the alternative expected to have the least ecological impacts.

**332 Oil spill response implications of fate and effect knowledge gaps for diluted bitumen pipeline spills** E. DeCola, Nuka Research and Planning Group. Several new projects have been proposed to transport tar sands oil in the form of diluted bitumen through North American pipeline systems. While pipelines in general are one of the safest means of oil transportation, it is impossible to completely eliminate oil spill risks. The nature of diluted bitumen presents challenges to oil spill responders, due to its physical and chemical properties. These challenges became apparent during the 2010 diluted bitumen pipeline spill in Marshall, Michigan. While that experience yielded some advances in diluted bitumen oil spill cleanup, significant knowledge gaps remain. Hydrocarbons behave in different ways when spilled to land or water, depending on the chemical composition and the environment to which it is spilled. It is critical to garner the best possible understanding of how a potential spill could behave in order to plan and prepare for an effective response. In particular, it is important to anticipate whether the spilled oil will float, submerge, or sink, and over what time periods and in what conditions. Sunken or submerged oils pose different environmental risks and require different oil spill containment and response strategies. In addition to challenges in predicting the fate and behavior of diluted bitumen spills, the actual cleanup of bitumen spills, particularly when the product sinks or submerges, is extremely cumbersome and often ineffective. The state of knowledge for responding to spills of diluted bitumen is still very immature. Containing submerged oil is more challenging than when the oil floats, and requires different equipment and techniques. Recovery of submerged oil on the bottom requires multiple systems for picking up the oil, separating the oil, water, and sediments, and treating the waste streams. Different techniques have been used, with varying success. Nearly all are extremely labor intensive. One commonality among most submerged oil detection, containment, and recovery methods is that they are extremely intrusive. Some techniques may actually cause more harm than good, and ecological risk assessment is a component of submerged oil response operations that should be addressed in the planning phases. This paper will explore the state-of-knowledge and technology for responding to diluted bitumen spills, and will identify significant knowledge gaps that impact oil spill response.

**333 Mitigation of Oil Spills** M. Gordon, M.R. Gordon & Associates Ltd. / Aquatic Resources & Env. Consultant; G. Bakker, M.R. Gordon & Associates Ltd.. The terms "mitigation" or "mitigate" are used often in environmental assessment (EA) or impact assessment reviews. In this context, mitigation is typically assumed to include cleanup of the spill, restoration of habitat and the regeneration of the native species that relied on the affected habitat. After participating in a number of EA reviews and reviewing the after-effects of various oil spills, it is apparent that references to mitigation may not adequately account for the scope of habitat conditions or the timeframe required to restore habitat and regenerate resources. This degree of uncertainty in turn directly impacts those that have relied on the affected resources. Watersheds vary widely depending on several variables: climate,



topography, water chemistry, soil chemistry and local meteorological conditions. This in turn relates to river flow, river substrate, lake depth and other characteristics that influence how an oil spill behaves in the environment. The diversity of habitat conditions that may be encountered when an oil spill occurs creates a high degree of uncertainty and poses significant challenges for cleanup strategies and engineering solutions. The ability to determine what restoration is needed depends on a good understanding of the resource abundance and diversity in the watershed. Using examples from oil spills, this presentation will discuss how effective mitigation strategies have been and what specific elements need to be considered to ensure that mitigation is successful. A basis for detailing the scope and timeframe for successful mitigation will be presented.

**334 Commemorating the 50th Anniversary of Silent Spring** B.A. Rattner, USGS-Patuxent Wildlife Research Ctr / USGS, USGS-Patuxent Wildlife Research Center / Beltsville Laboratory, USGS-Patuxent Wildlife Research Ctr / Beltsville Laboratory; N.H. Golden, U.S. Fish & Wildlife Service / Environmental Contaminants Program, U.S. Fish & Wildlife Service. The publication of *Silent Spring* in 1962 resulted in revolutionary changes in public opinion and government policy. Author Rachel Carson presented and discussed pesticide effects beyond target organisms. New technology for controlling pests had failed to give sufficient consideration to direct and indirect effects of pesticides on non-target invertebrates, fish, and wildlife, human health, and the environment. Carson described ecological imbalances, surface and ground water contamination, inadequate treatment of drinking water, persistence and trophic transfer of chlorinated hydrocarbons, use of natural products for biological control, human safety, and pesticide resistance. *Silent Spring* has been ranked by some as one of the 25 most important science books of the 20<sup>th</sup> century. It helped bring about new environmental laws and the establishment of government agencies to protect the environment. To some, Rachel Carson was one of the most influential Americans of all time, “the mother of the environmental movement”, and shaped the attitude of modern day society toward nature. To others, the charges made in *Silent Spring* lacked scientific rigor and created an unfounded fear of pesticide use that exists to the present day. The life of Carson, the content of *Silent Spring*, and the events and controversy that followed will be reviewed.

**335 Fifty years of POPs: Have we achieved our objectives?** K. Solomon, University of Guelph / School of Environmental Sciences, University of Guelph / Centre for Toxicology, School of Environmental Sciences. Environmentalism had its roots in ancient Greece where scholars, such as Hippocrates, linked diseases of humans to airs, waters, and places. However, this was in the absence of data and mechanistic understanding, science only arrived on the scene with the work of James Snow on cholera in London. Rachel Carson was one of the founders of modern environmentalism and the legacy that *Silent Spring* brought to the general public was an understandable explanation of the behavior of persistent and bioaccumulative substances in the environment. *Silent Spring* begat many progeny; the most globally important of these being the Stockholm Convention on Persistent Organic Pollutants (POPs), but this was not the first step in the science of POPs. Observational and experimental measurement of bioaccumulation in food chains was driven by better techniques of analysis and the use of radiolabelled tracers in the laboratory. The Stockholm Convention was grounded in the understanding of the classical POPs at that time (1995). These were the dirty dozen, the DDT, dioxins, furans, and other halogenated pesticides. These compounds were identified empirically on the basis of observed persistence, bioaccumulation, and toxicity (PBT). More recent programs have expanded the concepts that Rachel Carson highlighted to chemicals in general via TSCA in the US, the DSL in Canada, REACH in the EU and similar legal instruments in other jurisdictions. In this process we have learned that POPs do not have to share all the properties of the dirty dozen to present a risk to the environment. Persistence itself is not necessarily a ticket to a ban; this must be accompanied by partitioning into biota. Experience with the perfluorinated compounds in the environment showed that persistence is still key but that incorporation into the food chain does not require partitioning into lipids associated with compounds of high KOW. Even compounds with high KOW may not be problematic if other properties such as KAW result in partitioning into the atmosphere where rapid degradation occurs. We have learned to proactively instead of reactively but there is a danger that the pendulum will swing the other way

and we will be tempted to extreme precaution that will lead to the unnecessary regulation of too many false positives.

**336 50 Years after Silent Spring: Have Organophosphate and Carbamate Pesticides Met the Challenge?** A. Fairbrother, Exponent / EcoSciences, Exponent, Inc. / EcoSciences. Use of organophosphate and carbamate insecticides increased during the 1970s and 1980s, following the banning of DDT and reduction in use of organochlorine pesticides. Their short environmental half-lives and low chronic toxicity in people, plus the availability of a treatment antidote for accidental acute poisoning, made them a reasonable solution to some of the concerns raised in *Silent Spring*. However, birds are particularly sensitive to cholinesterase inhibitors and continue to experience acute poisonings. A large die-off of Swainson's hawks in Argentina in 1996 following a monocrotophos application for grasshopper control alerted the wildlife community to the potential for large-scale wildlife poisonings, either from direct exposure to spray or from eating contaminated prey. Other products, notably carbofuran, are used illegally to purposefully kill wildlife for predator control, crop protection, or food. There is concern that the cumulative use of multiple pesticides may affect populations of endangered salmon. These pesticides also may cause sublethal effects at low concentrations in both people and wildlife, such as acting as androgen receptor antagonists. Because these products are inexpensive relative to newer pesticides that have fewer nontarget effects, and because they are still effective for controlling a broad-spectrum of common agricultural pests, they remain an important component of large-scale agriculture. Rachel Carson noted “[We are] challenged as mankind has never been challenged before to prove our maturity and our mastery, not of nature, but of ourselves.” If we are to continue to use toxic, broad-spectrum pesticides, we can demonstrate our maturity by acknowledging that they are only one of a larger set of tools for integrated pest management (IPM); by treating them with respect as highly toxic substances and restricting access to only those who need them; and by maintaining an awareness of local wildlife vulnerabilities.

**337 Fate and Effects of the Phenyl pyrazole Insecticide, Fipronil, on Benthic Arthropods; What have we learned since Silent Spring?** D. Schlenk, University of California-Riverside / Department of Environmental Sciences. Fipronil is a phenylpyrazole insecticide that is currently used in a number of field and urban applications. The mode of action of it is based upon its arthropod-selective inhibition of the GABA chloride channel. Although this effect allows the compound to have relatively limited effects in mammals and vertebrates, impacts on target and non-target arthropods can be severe. One of the unique features of the molecule is that its transformation products have a similar or in some cases greater potency and have equivalent if not longer persistence particularly in sediments. Because of this behavior, benthic arthropods, particularly foraging detritivores, may be very susceptible to the acute and chronic effects of fipronil and its degradates. With regard to fipronil, significant progress has been made since *Silent Spring* with studies specifically focused on the chronic impacts of fipronil on arthropod populations and studies have incorporated stereoselectivity in the assessment of its fate and effects. However, understanding the potential for trophic and dietary exposure from sediment and detritus consumption by benthic arthropods is still under appreciated with regard to exposure assessments. *Silent Spring* supports the risk assessment of not only the parent compound but also pesticide degradates and forces us to better characterize the fate of fipronil and its degradates in the environment. And like DDTs and other OCs, it opened up the possibility that fipronil may have endocrine and chronic impacts to non-target organisms outside of simple acute toxicity.

**338 Nothing in Nature Exists Alone: Understanding the Effects of Pesticides on Amphibians in the Presence of Natural and Anthropogenic Factors** M.D. Boone, Miami University / Department of Zoology. When Rachel Carson published *Silent Spring*, she could rightly label many contaminants “elixirs of death,” because direct, lethal toxicity to non-target wildlife was readily apparent. Environmental concentrations of modern pesticides appear safer than early organochlorines, but the effects of modern pesticides are more subtle and can change with environmental conditions. Toxicity testing, however, focuses on lab studies designed for control in simplified environments, which does not account for important environmental variation that alters toxicity. Studies with amphibian communities in the lab and field have highlighted Carson's theme that “nothing in nature exists alone” in

that change in one species can have ramifications throughout the food web. For instance, insecticides can reduce sensitive members of a community like invertebrates, which can have divergent impacts on a community. Exposure to sublethal concentrations of insecticides can affect salamander larvae predation: invertebrate prey sensitive to contaminants may be eliminated or reduced by insecticides, while vertebrates like small tadpoles may persist with exposure, but suffer higher mortality through increased predation by salamanders. Alternatively, invertebrate predators of amphibians can suffer from insecticide exposure resulting in reduced predation, which can benefit amphibians through increased survival, which can consequently lead to greater levels of competition. Timing of exposure during the life cycle can also influence the impact of a pesticide with early life stages appearing to be most sensitive for some species, while others appear to subsequently suffer consequences of exposure later in life cycle. Finally, environmental factors, like temperature, ultraviolet radiation, presence of competitors or predators, presence of other pesticides, and amount of nutrients in the environment, can alter toxicity—in some cases changing the impact of the same concentration of pesticide from positive to negative. Regulation of pesticides on the road forward should involve incorporating important environmental factors into the regulatory process across multiple life stages. The ideal impact of pesticides to non-target wildlife is “no effect,” a goal that remains almost more elusive 50 years after Carson’s publication as we gain a greater understanding of the complexities with which a pesticide can influence individuals in a food web.

**339 Influence of Silent Spring on the U.S. Crop Protection Industry** S.R. Mortensen, BASF Corporation; J.D. Wisk, BASF Corporation / Ecotoxicology, BASF Corporation / Global Risk and Issues Management; M.F. Leggett, CropLife America; L. Guinessi, CropLife Foundation. The 1962 release of Rachel Carson’s book *Silent Spring* launched a public debate on chemical pollution, the use of pesticides, as well as mankind’s relationship with, and responsibility to, the world around us. The decade prior to the release of this book marked the beginnings of the Green Revolution in agriculture; a time when chemical use was responsible for unprecedented gains in pest control. When *Silent Spring* was published, the widespread use of synthetic chemicals for pest control was a relatively new practice. Industry’s initial reaction to *Silent Spring* was defensive, viewing the book as an attack on all pesticide use. But it was not. Rachel Carson advocated the integrated use of pest control methods, in harmony with nature in an era where use of technology to control man’s surroundings was ‘in vogue’. The debate regarding *Silent Spring* led in part to the creation of the U.S. Environmental Protection Agency and development of a regulatory system for pesticide registration and use. While many of the tools that would enable Ms. Carson’s vision to become a reality were already in development, current research and development efforts have shifted towards chemicals with more specific targets, lower persistence and lower environmental impact. Today pest control has embraced principles outlined in *Silent Spring* in the form of adoption of Integrated Pest Management strategies in forests, the public health sector and on farms. A strong network has developed for scouting pest pressure, and determining what needs to be sprayed when and where. Chemicals are rotated to manage resistance and reduce environmental impact. Efficiency advancements have been made in the targeting of applications. Integration of chemical herbicides with biological traits has enabled development of conservation tillage regiments on farms. The combination of agro-ecological knowledge with targeted inputs and seed technology has resulted in large gains in farm yields. These agricultural strategies have enabled North America to be a net food exporter while placing more land under conservation. Environmental gains since *Silent Spring* are due in large measure to formalized environmental policies, improved resource management, and the implementation of emerging technologies in pest control, which are all part of the enduring legacy of Rachel Carson.

**340 What effect do pesticides have on honey bee health?** R.M. Johnson, The Ohio State University – OARDC / Department of Entomology. Insecticides are, by definition, poisons intended to kill insects. The honey bee (*Apis mellifera*) is an insect, so it would seem logical to conclude that insecticides kill honey bees. This assumption is implicit in Rachel Carson’s “Silent Spring”, and this line of reasoning has led many people to assume that insecticides are the primary cause of Colony Collapse Disorder, the mysterious disappearance of honey bees first reported in 2006. However, the real relationship between honey bees and insecticides and other pesticides is far more complicated, and the issues have only become more complex since

“Silent Spring” was written. Modern analytical chemistry methods have demonstrated that a vast array of pesticides are present in bee hives, but the effects chronic low-level exposure to many different pesticides is not clear. Application methods, formulations and label restrictions have reduced bees’ exposure to the most toxic pesticides, but while the active ingredients in these formulations have changed, they are just as toxic to bees as the active ingredients used 50 years ago. The use of systemic pesticides has expanded, which protects bees from inadvertent contact exposure during pesticide application, but may lead to chronic oral exposure through pollen and nectar. Undoubtedly the biggest change is that beekeepers have started to apply pesticides directly inside beehives. With the introduction the *Varroa* mite, a devastating parasite of honey bees, beekeepers have begun using acaricides to stop these mites from killing honey bees. While honey bees clearly benefit from reduced parasitism after acaricide application, exposure to these pesticides may also affect bee health. In summary, insecticides continue to be toxic to bees, just as they were 50 years ago, and indiscriminate insecticide use continues to result in obvious mass killings of bees – though these events are rare. Instead, it is bees’ continual exposure to sublethal doses of many different pesticides, including pesticides applied by beekeepers, that may affect bee health in more subtle ways. Characterizing the effects of bees’ real-world pesticide exposure, that is chronic exposure to sublethal levels of mixtures of pesticides, is the challenging future of toxicological research on honey bees.

**341 Facilitating Management Decisions through Pesticide Sensitive Crops and Habitats Registry Outreach Programs** L. Hahn, Office of Indiana State Chemist / Water Quality/Endangered Species Program Specialist; L. Theller, Purdue University / Ag and Biological Engineering; B. Engel, Purdue University / Agricultural and Biological Engineering; A. Reimer, Purdue University / Natural Resources Social Science Lab, Department of Forestry and Natural Resources. The Driftwatch.org Pesticide Sensitive Crop and Habitats Registry was developed through a collaboration of producers of pesticide sensitive specialty crops, stewards of at-risk habitat (including pollinators) and pesticide applicator communities. The Drift-Watch Registry assists producers in mapping their field(s) and identifying their specialty crop type on the publicly accessible website [www.driftwatch.org](http://www.driftwatch.org). A second registry enables automated pesticide applicator notifications. Pesticide applicators can view sensitive fields and contact information in the area of their scheduled applications. Nearby habitats of endangered species are displayed, as are other themed data such as watersheds with public drinking water intakes. The goal of the developers of the registry is to facilitate effective communication between the specialty crop grower, pesticide applicator and stewards of surrounding at risk habitat. This presentation will highlight (1) current measures of website communication effectiveness of participating states; (2) social science survey outcomes measuring attitudes, motivations and behaviors of commercial pesticide applicators towards natural resource concerns and use of the website; and (3) selected examples of (state) progress in the management of pesticides.

**342 Characterizing soil-to-vegetation transfer factors (TFs) and quantifying uncertainty for uranium TFs in Grand Canyon area terrestrial habitats** G. Linder, USGS/BRD/Columbia Environmental Research Center, HeronWorks Field Office, USGS/BRD/CERC / HeronWorks Field Office; J. Hinck, USGS/BRD/CERC; S. Finger, USGS/BRD/CERC, Geological Survey NRDAR Coordinator / Columbia Environmental Research Center; E.E. Little, USGS/BRD/CERC, Ecology Branch Chief / Columbia Environmental Research Center; D.E. Tillitt, U.S. Geological Survey / Columbia Environmental Research Center, USGS / Columbia Environmental Research Center. Exposures of terrestrial biota to naturally occurring uranium have reappeared as a concern in northern Arizona adjacent to Grand Canyon National Park (GCNP). Whether we consider exposures of terrestrial biota to uranium linked to soils or atmospheric dusts, relationships between these exposure matrices and vegetation are generally characterized by relatively few empirical data. Furthermore, we are uncertain of the relationships between TFs for uranium that are frequently applied in the literature, or to those TFs characteristic of soil-vegetation systems in the vicinity of GCNP. Here we focus on a nested analysis of TFs compiled from existing literature and project-specific uranium concentrations in soils collected from locations near GCNP. Based on this nested analysis, we characterized soil-to-vegetation TFs used for screening level evaluations of risks linked to dietary exposures to uranium in terrestrial biota typically encountered in habitats of GCNP and environs. Soil-to-vegetation TFs are simple ratio estimators that describe the

relationship between the concentrations of a constituent, here, uranium in soil and the concentrations of that same constituent in vegetation growing in that soil. To characterize uranium TFs for areas in the vicinity of GCNP, we completed a literature search and compiled existing data for soil-to-vegetation TFs for uranium, then assembled various data subsets sufficient to characterize TFs based on geographic or vegetative characteristics. Simple distribution plots and distribution-fitting methods were initially used to graphically characterize these data assemblages. Then, we estimated uranium soil-to-vegetation TFs using tools from exploratory data analysis, as well as bootstrap derivations of 95% confidence interval (CI) for 50%-tile estimates of TFs and bootstrap-implemented quantile regression estimates of 50%-tile with 95% prediction intervals (PI). For example, bootstrap estimates of median TF values were 0.042 (CI: 0.026-0.15), whereas quantile regression estimates for TF median values were 0.042 (PI: 0.029-0.10). Thus, if soil U concentration was 50 mg U/kg dry weight we would estimate a concentration of 2.1 mg U/kg dry weight in vegetation. The quantitative uncertainty estimates of TFs, if incorporated into dietary exposure equations, can contribute to sensitivity analyses subsequently completed during characterization of risks associated with uranium exposures in soils or dusts under field settings.

**343 Derivation of soil thresholds to protect chisel-toothed kangaroo rat from uranium mine waste in northern Arizona** J. Hinck, US Geological Survey / Columbia Environmental Research Center; G. Linder, US Geological Survey; J. Otton, US Geological Survey (retired); S. Finger, E. Little, D. Tillitt, US Geological Survey. The ecotoxicological risks of uranium mining in the Grand Canyon region are largely unknown, and chemical concentration data in biota inhabiting the region is lacking. Many species such as the Arizona chisel-toothed kangaroo rat (*Dipodomys microps leucotis*) have specialized life history strategies and physiological adaptations that allow them to survive in the region's arid environment but may also increase their exposure to mine-related contaminants. Chemical data from soils and waste rock samples collected from five uranium mines (3 reclaimed, 1 left on standby and not reclaimed, and 1 never mined) were used in a screening level risk analysis. Dietary toxicity reference values based on lowest observed adverse effect levels from the scientific literature were used to estimate soil thresholds representing risk to kangaroo rats. Concentrations of arsenic, cadmium, copper, lead, nickel, thallium, uranium, and zinc were approximately an order of magnitude greater in soils at the non-reclaimed mine than those from reclaimed mines. Sensitivity analyses indicated that body weight critically affected outcomes of exposed-dose calculations; juvenile kangaroo rats were more sensitive to the inorganic chemical toxicities than adults. Given the natural history of kangaroo rats, consensus-based toxicity thresholds (e.g., EcoSSLs) were overly conservative and overestimated risk. Therefore, we derived soil thresholds to protect kangaroo rats for arsenic (273 mg/kg), cadmium (39 mg/kg), copper (1827 mg/kg), lead (2286 mg/kg), nickel (3854 mg/kg), thallium (6.4 mg/kg), uranium (1513 mg/kg), and zinc (3653 mg/kg) using toxicity reference values that more closely replicate field exposure of kangaroo rats. Inorganic contaminants in soils in and near the mine areas generally posed minimal risk to kangaroo rats. Most exceedences of soil thresholds were for arsenic and thallium and were associated with mine waste rock; few exceedences occurred for cadmium, copper, lead, nickel, uranium, and zinc. Management goals to protect kangaroo rats from arsenic may also protect them from the individual chemical toxicities of other inorganic constituents but would not account for additive, antagonistic, or synergistic effects of chemical mixtures. Studies to establish chemical toxicity thresholds using kangaroo rats and other burrow-dwelling desert mammals are warranted to better characterize risk to species that have evolved and thrived in desert conditions.

**344 Estimating population exposure to ionizing radiation caused by the Fukushima Daiichi nuclear disaster** M. Takagi, National Institute for Environmental Studies; A. Takeuchi, National Institute for Environmental Studies / Central Research Laboratory; A. Tanaka, H. Nitta, National Institute for Environmental Studies; S.F. Nakayama, National Institute for Environmental Studies / Centre for Environmental Health Sciences, National Institute for Environmental Studies / Center for Environmental Health Sciences. The 9.0-magnitude undersea megathrust earthquake occurred on 11 March 2011 in the north-western Pacific Ocean with its epicenter approximately 70 km east off coast of Tohoku, Japan. The earthquake triggered huge tsunami waves that reached heights of up to 40 m and travelled up to 10 km inland. The earthquake and tsunami caused more than 15,000 deaths

and 27,000 injured with over 3,000 people still missing, which also brought substantial economic impacts. The destructive tsunami waves disabled the reactor cooling systems, resulting in nuclear radiation leaks, hydrogen explosions and partial nuclear meltdowns. This largest nuclear disaster since Chernobyl of 1986 released significant amounts of radioactive materials, including iodine-131, caesium-134 and caesium-137, into the atmosphere and ground/ocean waters. Radioactive fallout caused significant public concern, leading voluntary but unnecessary evacuation, reputation risk of locally produce foods and delay in disaster waste disposal. The Japanese government and other governmental and academic institutions carried out measurements of radioactivity nationwide, of which data are available through many web sites. However, to date systematic exposure assessment has not yet been performed to our knowledge. In order to perform comprehensive risk assessment, it is important to estimate general population's exposure to ionizing radiation caused by the nuclear disaster. In this study, built was a model combining external and internal radiation exposure. For external exposure, the model included airborne radiation measurement data, radiation shielding rate and time spent outside by each age category. The internal exposure model consisted of inhalation doses, market-basket data, food consumption rate, dust and soil data and behavioural patterns of each age category. Using SAS "rand" function, performed was Monte Carlo simulations with 500,000 iterations. The model revealed no one had external exposure to more than 1 mSv during the first year since the disaster except Fukushima Prefecture (median < 0.1 mSv). Further details of the model and its result will be shown in the presentation.

**345 Gamma radiation-induced damages in the monogonont rotifer, *Brachionus koreanus*** J. Lee, Hanyang University / Dept of Chemistry, Hanyang University Graduate School / Dept of Chemistry; J. Han, Hanyang University. To investigate the impacts of gamma radiation on aquatic organism, we tested the monogonont rotifer, *Brachionus koreanus* as a model species. Several parameters including 24-h toxicity test, population growth rate, and intracellular reactive oxygen species (ROS) level were measured after exposure to a wide range of gamma radiation dose. To check the transcriptional change of important inducible genes such as glutathione S-transferase (GST) genes, heat shock protein (Hsp) genes, DNA repair-related (e.g. replication protein A; RPA, DNA dependent protein kinase; DNA-pk, Ku70/80) and DNA damage response genes (e.g. p53, Bcl-2) after gamma radiation, we measured dose- and time-dependency at 0, 50, 150, 200 Gy by using the quantitative real-time RT-PCR. Results showed that gamma radiation induced gradual growth retardation of *B. koreanus* at 150, 200 Gy. But 24-h toxicity test of *B. koreanus* was not observed at all doses. The level of ROS was high at 50, 100, 150, 200 Gy, suggesting that all the doses would be already toxic. The expression of GSTs, Hsps, DNA repair-related and DNA damage response genes mRNA was up-regulated in a dose- and time-dependent manner. Of all genes, RPA1 and p53 transcripts levels were observed up to about 50 and 40 folds relative to the control 1h after gamma radiation (200Gy), respectively. This result indicates that gamma radiation can induce growth retardation with up- or down-regulation of important genes in DNA replication, chaperoning and repair process. Our studies are helpful in expanding knowledge of the molecular mechanisms on impacts of gamma radiation in aquatic organisms.

**346 Long-term fate simulation of radioactive cesium by multimedia fate model in Fukushima region in Japan** N. Suzuki, Y. Imaizumi, National Institute for Environmental Studies; Y. Morino, National Institute for Environmental Studies; T. Ohara, National Institute for Environmental Studies. Long-term multimedia fate of radioactive cesium species in Fukushima and surrounding region in Japan is simulated by multimedia fate model methodology. Radioactive nuclides emitted into atmosphere by the accident of Fukushima nuclear power plant were transported in the atmosphere from the plant to the Fukushima and surrounding region within a few hours after the emission. Part of transported radionuclides, especially those with lower volatility are considered to be deposited onto surface through wet and dry deposition processes. In this study, long-term fate of radioactive cesium species in the region after the short-term atmospheric transport is simulated by the combination of atmospheric transport model CMAQ and multimedia fate model G-CIEMS, on the former which is already reported by co-authors in elsewhere. Here the latter of the process after deposition of radioactive cesium species onto surface is reported. Accumulation of cesium species in soil, transport of them to the river through erosion and runoff processes, transport by river flow networks and fate between their sediments,



similar processes in lakes and sediments, and final output to sea are connected by the G-CIEMS. Based on the original feature of the G-CIEMS, GIS-based real river network segmented by approximately 5 km paths and their topological connections are used to simulate the transport along river flow. Runoff from river catchment to river segment is calculated based on the runoff processes in combination of soil erosion and water runoff. Using this simulation framework, long-term fate of levels of cesium species during 20 years after approximately 1 month emission period is simulated in soil, river water and sediments, and fluxes among those media. As a result, it is revealed that total amount of cesium species existing in soil compartment could be declined slightly faster than expected radioactive decay, because of the impact of runoff processes. Levels in soil and river sediments are simulated roughly comparable ranges compared to the observed levels. Amount existing in river networks is small compared to the soil compartment; however, the declining trend of the media might be slower than soil compartments, maybe because of the combined mass-balance impact of runoff, sedimentation, partitioning and outflows from the river system. Partitioning parameter of cesium species between soil and water may have major impact on the runoff processes of cesium in the regional fate system.

**347 Providing access to environmental radioactivity measurements during crisis and in peacetime : two tools developed in France by IRSN** E. LEPIEUR, Institute for Radioprotection and Nuclear Safety / Radiation Protection Division; g. Manificat, IRSN-Institute for radioprotection and nuclear safety / Environmental radioactivity study and monitoring department. All over the French territory, thousands of radiological environmental measurements are carried out each month, whether by IRSN, operators of nuclear facilities or other public, private or non-governmental organizations. In the event of a radiological accident, many additional measurements would also be performed. This multiplicity of actors makes data collection difficult and consequently these monitoring data are not easily accessible to experts and policy makers, but also to the general public. To solve this problem, two projects were launched by IRSN with the aim of developing tools to centralize information on environmental radioactivity in normal situation (RNM project for "Réseau National de Mesures" – National network of radioactive measurements) and during a radiological crisis (CRITER project for "CRIse et TERrain" - Crisis and field). The RNM network is created to contribute to the estimation of doses from ionizing radiation and to inform the public. To achieve this goal, the network collects the results from the different French stakeholders of environmental radioactivity measurements obtained in normal situation. In addition the RNM network is accessible for the public through the Internet. In the event of an radiological emergency, IRSN's mission is to centralize and manage – at national level – all the results of measurements or analysis performed by all the stakeholders, in order to accurately and permanently determine the radiological state of the environment, before, during and after the accident. The CRITER project involves data collection from all potential sources, their transmission, their organization, and the publication of the measurements during the crisis or post-accident situation. For all events with possible radiological health and environmental consequences requiring the activation of an emergency response organization, a specific CRITER database is created. For example, a CRITER IT system was implemented after the Fukushima accident in March 2011. Both projects have developed complementary tools with compatible data repositories, allowing not only to form a national reference database with measurements taken in normal situation from all environmental compartments and around each nuclear facility, but also a crisis database able to quickly centralize the new measurements available from all those on the affected area and give access to these data for all decision-making bodies.

**348 Summary of field ecological studies conducted to assess exposure to radionuclides at the Los Alamos National Laboratory** R.T. Rytz, Neptune and Company, Inc.. Field studies have been used as one line of evidence to assess the potential for ecological risk from radionuclides and other contaminants. This paper summarizes field studies conducted at the Los Alamos National Laboratory (LANL) in New Mexico, USA. Although both terrestrial and aquatic studies have been conducted at LANL, this paper summarizes information on the terrestrial environment. Key radionuclides measured in LANL plants and animals are cesium-137, strontium-90, plutonium-238, and plutonium-239/240. Uranium has been measured as a metal and as isotopic uranium (uranium-234, uranium-235, and uranium-238). Wildlife studies have focused on the small mammal community although some broad-ranging mammals (like elk and deer) or birds have been sampled.

Some of the studies have evaluated tissues separately from pelts to better understand exposure pathways and contaminant transport. It is important to understand the spatial scale of the contamination, the residence time of the wildlife and their home range to interpret the results of such studies. Lastly, recommendations for improvements to plant and wildlife radionuclide monitoring are presented.

**349 Tissue radionuclide concentrations in water birds and upland birds on the Hanford Site (USA)** D.A. Delistraty, Washington State Department of Ecology / Hazardous Waste and Toxics Reduction Program; S. Van Verst, Washington State Dept. of Health. Historical operations at the Hanford Site (Washington State, USA) have released a wide array of non-radionuclide and radionuclide contaminants into the environment. As a result, there is a need to characterize contaminant effects on site biota. Within this framework, we evaluated radionuclide concentrations in bird tissue, obtained from the Hanford Environmental Information System (HEIS). The database was initially sorted by avian group (water bird [WB] vs. upland bird [UB]), radionuclide (over 20 analytes), tissue (muscle, bone, liver), location (onsite vs. offsite), and time period (1971-1990 vs. 1991-2009). For this presentation, the purpose of our study was to compare concentrations of Co-60, Cs-137, and Sr-90 in WB vs. UB on the Hanford Site, as well as to compare Sr-90 concentrations in muscle vs. bone in birds at Hanford. Onsite median concentrations in WB were significantly higher (Bonferroni  $P < 0.05$ ) than those in onsite UB for Cs-137 in muscle (1971-1990) and Sr-90 in bone (1991-2009). These observations may be linked to behavioral, habitat, or trophic differences between avian groups. As expected, median concentrations of Sr-90 in bone were significantly higher (Bonferroni  $P < 0.05$ ) than those in muscle for both WB and UB. This presumably relates to Sr-90 serving as a calcium analog, preferentially depositing in bone. Overall, our results contribute toward ongoing efforts to characterize ecological risk at the Hanford Site.

**350 NOAA Talks Turtle** M. McDaniel, McDaniel Lambert Inc.. BP's oil flowed unchecked for over 105 days after the tragic Deepwater Horizon explosion that killed 11 workers on April 20, 2010. The oil soiled the waters, shores, livelihood and sense of well-being of those who lived and worked in five states on the Gulf coast. The 24-hour Internet news cycle, awash with twitter feeds, independent bloggers and professional journalists flooded the waves with verified and unverified news, pictures and videos. BP's every move was broadcast at high speed and in full color, revealing their lack of crisis management as they stumbled through high-scale efforts to stop the leak. It was within this climate that a little known branch of the National Oceanic and Atmospheric Administration (NOAA) scientists began to explain their incredibly technical process. This group manages the National Resource Damage Assessment (NRDA) program that assesses ecological damage and loss of human use of natural resources. Although ultimately geared to "make the stakeholders whole," NRDA's very structure—a multi-year, multi-trustee, polluter cooperative and lawyer-heavy process—worked against communication. Realizing they were hopelessly technical, the smart NOAA scientists were quick to identify their crisis communication weakness would be more of an Achilles heel in this process than NRDA itself. They undertook a series of risk communication trainings to better prepare team members for community outreach. Ultimately NOAA scientists learned their success relied on not hiding behind the data. They developed techniques to explain technical information clearly and accurately to communities of non-technical, outraged, and disadvantaged stakeholders. They gained insights into dealing with outrage and how risk perception magnifies or diminishes risk. They consistently got information out promptly. The scientists also learned how to let their passion for the natural resources of the Gulf become the bridge that connected them to local communities. NOAA couldn't change the arduous legal process or scientific rigor of NRDA, but they worked hard to earn trust and credibility with a skeptical public. And it worked.

**351 An Education Initiative Program as a Risk Communication Tool at TVA Kingston** S. Young, ARCADIS, ARCADIS / Environmental; D. Jones, R. McKinney, ARCADIS; N.E. Carriker, TVA Kingston Ash Recovery Project / Kingston Fly Ash Recovery Project. The December 2008 Kingston ash release discharged approximately 5.4 million cubic yards of coal slurry into the surrounding environment. This event raised concerns of resident health and safety within the community and spawned predictions of dire effects on the aquatic ecology as a result of bioaccumulation

of ash-related constituents. As TVA worked with federal and state agencies to clean up and restore the area surrounding the Kingston Fossil Plant, the agency interacted with local, state, and Federal (USEPA) officials to create a forum for residents to voice their concerns and provide input into the cleanup process. TVA also recognized the opportunity to contribute to the academic and personal growth of local students. As a result, TVA developed an Educational Initiative to inform students about the scientific investigations that were taking place at the release site. The Educational Initiative included three separate information sessions presented to more than 1,000 students at high schools in Roane County, TN. These were designed to teach students about the causes of the spill; describe the immediate response and on-going efforts to repair the damage; and introduce the engineering and scientific disciplines being applied to the cleanup and environmental monitoring that will continue for years to come. Students participated in hands-on lessons about chemical elements that affect their daily lives, the air they breathe and the water they drink. They also learned about various animals, fish and bugs that comprise the food web, as well as the materials and methods scientists use when studying living creatures. This presentation highlights some of the lesson material used in the sessions, including video clips recreating the initial failure, live footage of different sampling methods used for abiotic and biotic media, and quizzes given to students to test their knowledge. This highly successful program helped this generation of students better understand the event and how it relates to their daily lives. Further, the Education Initiative helped foster the relationship and trust between TVA and the affected community, which is the cornerstone of risk communication.

**352 Fishing for S.T.E.M. Literacy Initiative: A National Science Foundation Proposed Project for Increasing Informal Science Education** R.S. Grippio, Arkansas State University / Department of Biological Sciences, Graduate Program in Environmental Science; A.A. Gill, Arkansas State University / ASU Center for Digital Initiatives; R. Johnson, Arkansas State University / Biological Sciences; J.T. Kennon, Arkansas State University / Dept of Chemistry & Physics; T. Risch, Arkansas State University / Department of Biological Sciences. The National Science Foundation (NSF) Directorate for Education and Human Resources is tasked to build a globally competitive, diverse science, technology, engineering and mathematics (STEM) workforce that will engage the public as science learners, allowing citizens to address societal changes including environmental challenges. The NSF Informal Science Education (ISE) program is part of this Directorate and supports innovation in anywhere, anytime, lifelong learning outside formal school settings through investments in research, development, infrastructure and capacity-building. Promotion of a STEM workforce is especially important to SETAC because these are the disciplines from which most members are drawn. Thus, developing informal science learning in STEM areas will increase the awareness and support of SETAC mission and activities. Fishing is a popular recreational activity in Arkansas. However, many participants do not have the background knowledge required to understand the complex STEM concepts underlying a healthy aquatic system and sustainability of the natural resources they are using. To address this, we propose the Fishing for STEM Literacy ISE initiative utilizing an angling platform. After a year of planning, the implementation of five pilot studies will be undertaken to collect data and allow program evaluation. This will include pre- and post-survey data derived from paper and virtual environments. These will evaluate the understanding of STEM concepts underlying the sustainability of healthy aquatic resources and fisheries, attitudes toward science and the environment, parental perceptions of their children's STEM abilities, and levels of involvement in fishing and water quality activities. This program builds on existing knowledge and interest in a rural youth population to increase understanding of STEM concepts including food chains, water quality and chemistry, aquatic life, population biology, taxonomy and conservation of resources. A target audience of 10-15 year olds and their parents will foster career aspirations in children at an age at which commonly there is a departure from STEM career interests.

**353 Working Towards Agreements: Three Rules for Effective Risk Communication** A. Chun, The Chun Group. The goals of risk communication range from successfully relaying complicated technical information to gaining the public's approval of decisions based on complicated technical information. The latter is the subject of this presentation and is a complex negotiation or risk management process. Public approval depends on the ability of a risk communication program to inform, engage, and respond.

Successful risk communicators require a negotiator's attitudes and skills for identifying and understanding the interests of different stakeholders and for building trusting relationships. One must also appreciate how different perspectives can influence risk perception and people's willingness to negotiate. Three key rules are: Expect the unexpected and know how to respond effectively. Discuss difficult problems privately first. Always be nice and respectful. For resolving ecological or human health problems, the differences are in the stakeholders and their interests, but all three rules apply. An example from a risk communication effort involving the USEPA's Superfund Program will be used to demonstrate this.

**354 Use and misuse of Risk-Based Screening Levels** A. Guiseppe-Elie, DuPont Company / Corporate Remediation Group; E. Quinn, US Environmental Protection Agency / Region III; T. Bingman, DuPont Company / Corporate Remediation Group; R.G. Stahl, DuPont Company / Corporate Remediation Department, DuPont Company / Corporate Remediation Group, DuPont Company / DuPont Corporate Remediation Group. Risk-based Screening Levels (RSLs) have been developed for various media (e.g., surface water and biological tissues). These values are important in a tiered approach to human and ecological risk assessments as they serve a triage purpose in determining which potential exposure situations need further evaluation versus those that are of lesser concern. RSLs tend to be calculated in a conservative manner in a way that affords protection to sensitive human and ecological receptors for the particular exposure scenario. However, RSLs can be misused. In this case, we highlight why a fish-tissue mercury screening level should not be applied to waterfowl, and offer an alternative approach that would be more appropriate for determining potential exposure/risk concerns for hunters. Waterfowl might be a potential mercury source to hunters because of their proximity to the water bodies where mercury contamination exists. While it might be tempting to apply fish-tissue screening levels to waterfowl, the derivation of these are based on significantly different exposure assumptions than appropriate for waterfowl hunters. For example, EPA's water quality criterion is set to be protective of the general population, includes other dietary and non-dietary sources of mercury, and assumes a daily food ingestion rate that is inconsistent with hunting patterns. While, it is appropriate to consider the potential for exposures to hunters who may consume waterfowl, this evaluation is best done on a site-specific basis rather using a fish advisory level.

**355 Post Mortem of an Effective Risk Communication Effort – The Sierra-Crete® Case** T. Bingman, A. Guiseppe-Elie, DuPont Company / Corporate Remediation Group; R.G. Stahl, DuPont Company / Corporate Remediation Department, DuPont Company / Corporate Remediation Group, DuPont Company / DuPont Corporate Remediation Group. Effective risk communication can occur only once an open and honest dialogue between stakeholders develops. Risk communication pioneers, such as Sandman and Covello have described the concepts of outrage factors and source-message-channel-receiver models, respectively, as important elements in this discipline. By anticipating potential outrage factors within the receiving community and developing an approach to openly engage community stakeholders, it is possible to develop a risk communication strategy that effectively meets the needs of all parties. For this presentation, we provide a post mortem of such an example using Sierra-Crete®. This road sub base material, which had been used in several residential communities, was shown to contain trace levels of dioxins and furans. Following a risk assessment that demonstrated a lack of health risks in the community, the challenge of informing the community of the situation began. In this case study, we will examine how outrage factors such as dread, trust, control and children's issues can all be effectively addressed to contribute to the success of the risk communication effort.

**356 Environmental Awareness and Public Communications at U.S. Army Aberdeen Proving Ground: 35 Years of Lessons Learned** M. Ciarlo, EA Engineering, Science, and Technology, Inc., EA Engineering, Science, and Technology; K. Stachiw, Kenneth Stachiw; J. Weintraub, U.S. Army Aberdeen Proving Ground Department of Public Works. The U.S. Army Aberdeen Proving Ground has been a center for testing, training, and research since 1917. It has been home to landmark programs in the nation's defense, many of which were conducted prior to the current era of environmental awareness. APG is located alongside the Chesapeake Bay and contains thousands of acres of wetlands, forests, and natural resources. The environmental management history at APG is overall a success story,

with decades of work with hazardous materials and waste offset by decades of intense environmental scrutiny and protection. This history includes landmark environmental projects and policy with unique and precedent-setting challenges and solutions. The past 100 years – and especially the last 35 – at APG provide lessons learned that are of great potential and may help inform future environmental management. This presentation focuses on the relationship that has developed over the past 35 years between APG and its stakeholders for hazardous waste and environmental clean-up. These years represent a period of intense change during which significant effort was put forth to develop collaboration and cooperation in addressing hazardous waste issues. This presentation briefly outlines this aspect of APG's history and identifies a number of important lessons learned.

**357 Methods and Tools of Effective Communication during Emergency Response Actions** P. Kurzanski, CSX Transportation; J.R. Clarkson, Arcadis U.S., Inc. / Senior Vice President, ENVIRON, ENVIRON. September 28, 2012 marks the 30-year anniversary of the Livingston Train Derailment. This has been called the 'worst chemical transportation accident in the state of Louisiana history' and also 'the best managed chemical transportation accident.' For two weeks, the energies and expertise of eleven state agencies, six federal agencies, and five non-governmental entities, as well as the civic resources of the town of Livingston and neighboring communities, were focused on the mitigation of this chemical transportation accident. Protection of the health and welfare of human and ecological life and the effective management of the emergency were outcomes of federal and state legislative action and comprehensive hazardous materials spills emergency planning within the state of Louisiana. The effective management of this environmental crisis was documented in a film produced by the United States Environmental Protection Agency, which was used for many years to train first responders at the Environmental Response Team center in Edison, New Jersey. Many of the lessons learned during this incident have also been incorporated into the federal emergency management system for mitigating chemical emergencies in the United States. Communication and education about human health and ecological risk were critical to the effective management of this emergency response. Through the use of this example and several others, the presentation will highlight methods and tools of communication that were effective 30 years ago and have stood the test of time and are still effective today. This presentation will also highlight several methods and tools that are no longer successful given the explosion of internet access, social networking sites, and 24/7 news cycles of recent years. Possible alternative methods to meet these communication challenges will also be presented.

**358 Incorporating bioavailability into risk assessment for metal mixtures: Results of a comparative evaluation** E.J. Van Genderen, International Zinc Association / Assistant Manager, Environment, International Zinc Association / Manager, Environment & Sustainability; E.C. Rogevich-Garman, NiPERA / Ecotoxicologist; R.L. Dwyer, International Copper Association; J. Gorsuch, Copper Development Association. While the concepts encompassing mixture toxicity and modeling have been around for decades, only recently have new approaches ("bioavailability models") been expanded to consider metal mixture scenarios. Although current environmental regulations rarely require assessment of chemicals mixtures, the metals industries consider research on mixtures in the environment and development of efficient and economic science-based risk assessment approaches for metals mixtures essential to preparing for future regulatory demands and vital for ensuring adequate environmental protection. To this end, a comparative modeling evaluation was commissioned by the copper, nickel and zinc industries in an attempt to compare and contrast the available approaches for modeling metal mixtures in fresh water using the principles of bioavailability. Four international research groups, who are actively developing, testing and publishing a range of bioavailability-based approaches, were each provided the same mixture dataset (nearly 2500 metal mixture and associated single-metal exposures) to which they applied their respective modeling approaches. The dataset included toxicity exposures for seven metals in various binary, ternary and quaternary combinations using both spiked (laboratory and field waters) and ambient (field waters) experimental designs. Results of the comparative evaluation demonstrated that despite representing the spectrum of modeling complexity, the various modeling approaches each provided consistent conclusions concerning interaction types and importance of accounting for bioavailability in metal mixture risk assessments. Similarly, all of the modeling approaches

were challenged by metal combinations where significant deviation from concentration addition hypotheses occurred. Overall, approaches have been successfully developed to incorporate bioavailability into metal mixtures risk assessment. In addition, this evaluation illustrated the importance of incorporating variability into effects predictions and the need to emphasize the science behind modeling biological complexity when communicating risk.

**359 From substance- to societal solutions in risk assessment and sustainable risk reduction** L. Posthuma, RIVM / Lab. for Ecological Risk Assessment, RIVM / LER; D. De Zwart, RIVM / Lab. for Ecological Risk Assessment; D. Sijm, J. Verhoeven, RIVM / Substances Expertise Centre; S. Dyer, Procter & Gamble; K. Kapo, Montani Run, LLC; A. Burton, University of Michigan / School of Natural Resources & Environment and Cooperative Institute for Limnology & Ecosystem Research. Environmental quality criteria are critical in ecological risk assessment and risk remediation of chemicals. But: criteria exceedance signals regulatory problems and a potential for impacts, but not problem-solving solutions. Situations are multi-faceted: acute or chronic exposures, habitat alterations and presence/absence of refugia may occur, to mention a few. To illustrate that a multi-pronged solution-oriented approach works, we provide 3 case studies. First, in chemical regulation (e.g., REACH), PNEC-exceedance for a compound triggers a restriction evaluation of that compound, with optional replacement by an alternative chemical. Restriction follows when the use of the alternative results in lower impact. The final solution involves an evaluation of the trade-offs of chemical performance, information needs and risk per use category. Second, chronic impacts of multiple stress, including mixtures of toxicants, are to be expected within river catchments. Emissions of agriculture, industry and waste waters cumulate, while other stressors also vary. Impact diagnostics help to optimize impact reduction measures, brought together in river basin management plans. By developing innovative, landscape-level diagnostic techniques, the impacts of mixtures can be disentangled from influences of habitat deterioration, which improves the efficacy of river basin management. An Ecosystem Reality Check needs to be made before focus is put to a single stressor. The final solution involves pragmatic yet synthetic river basin management. Third, natural disasters occur, partly associated to massive unintended release of chemicals. Our common 'safe concentrations' need be replaced by 'impact distances', since the latter are key to prioritize impact reduction efforts in disaster events. Impact distances are needed quickly for direct exposures of man as well as for the environment, the latter related to ethical issues as well as to key ecosystem services to man, such as the provision of food. An approach to provide these distances has provided a pragmatic and implementable solution that optimizes impact reduction for humans and ecosystems. Solution-oriented risk assessment triggered innovative steps in risk assessment, partly re-using existing methods. Relevance of this way of working will be illustrated via the cases. Surprisingly, an unintended extra use has evolved: the safe distance model has improved disaster preparedness around the globe.

**360 Environmental Risk Assessments: The Value in Using Decision Analytic Methodologies** C. Stahl, U.S. EPA Region III, U.S. Environmental Protection Agency, Region III / Environmental Assessment and Innovation Division, U.S. EPA Region III / Environmental Assessment and Innovation Division, U.S. EPA Region III / Air Protection Division; J. Babendreier, U.S. Environmental Protection Agency / Office of Research and Development; A. Cimorelli, U.S. Environmental Protection Agency, Region III; J. Kremer, U.S. Environmental Protection Agency, Region III / Environmental Assessment and Innovation Division. Many current approaches often implicitly integrate many different pieces of information to arrive at an assessment conclusion or actionable decision without taking advantage of more transparent and robust decision analytic methodologies. Therefore, even when explicit decision making is not a goal, the integration of multiple criteria to create a biological index or arrive at an assessment conclusion can benefit from the use of good decision analytic techniques. This presentation will describe the feasibility of robust analyses using currently existing technologies and concepts using specific applications as examples – and the benefits of applying decision analytic techniques to environmental assessments that require integration of multiple endpoints and the potential dangers of using un-integrated or implicitly-integrated analyses in arriving at assessment conclusions. Current commonly-used approaches tend to focus on single disciplines or media, even when the larger environmental system is acknowledged, and present these narrowly-focused assessments as complete assessments of the environmental problem. This presentation will include



examples where the environmental assessment can support alternative conclusions even when the same data, but differently organized and differently valued, are used. Data, model and decision uncertainty impact the environmental assessment conclusions and these can be explored through the use of decision analytic methodologies; providing assessment analysts and decision makers a more robust analysis. Disclaimer: Although this work was reviewed by EPA and approved for publication, it may not necessarily reflect official Agency policy. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

**361 The need for more ecological realism in Ecological Risk Assessment procedures** M. Vighi, University of Milano Bicocca / Department of Environmental Sciences, University of Milano / Department of Environmental Sciences. In the last few decades, ecological risk assessment was based on simplified tools in order to provide methods suitable to be applied in support of chemical regulations. This pragmatic approach produced transparent and easy methods, applicable using the reduced information available, and allowed the development of many international regulatory tools. Chemical control in the last few decades substantially changed environmental and pollution problems in developed countries. In spite of their practical usefulness, these tools, mainly based on laboratory toxicological tests, suffer for a lack of ecological realism. Their capability to describe and predict the actual consequences on structure and functions of natural ecosystems is poor. Therefore, there is the need for the development of new tools capable to accounting for the complexity of communities and ecosystems. In the frame of scientific committees of the European Commission (SCHER, SCENHIR, SCCS) a Working Group is ongoing on "Addressing the New Challenges for Risk Assessment". The environmental subgroup highlighted a number of relevant issues worth to be developed: Increasing the ecological realism of exposure assessment. Assessing the effects of highly time-variable exposure. Covering the gap between effects at cellular or individual level and consequences for ecologically relevant end points. Assessing the vulnerability of natural biological communities and ecosystems. Assessing the role of indirect ecological effects of stressors. Assessing the interactions between combined stressors and environmental factors. Developing Trait-based risk assessment. Improving Ecological Modelling. Improving the scientific bases for the development of extrapolation approaches. A short overview is given on the main issues and on their realistic applicability (in the short, medium and long time) in risk assessment procedures for regulatory purposes.

**362 The future of risk assessment at regional scales with multiple sources, stressors and endpoints in the 21st century** W.G. Landis, Western Washington University, Institute of Environmental Toxicology. Risk assessment in the last century primarily consisted of a single stressor, usually a chemical, acting on a few endpoints at relatively small scales. Often the scale was limited to a contaminated site, although some of these were relatively large. The methods used were often simple risk quotients although some investigators did pioneer the use of spatially explicit techniques. In the early part of the 21<sup>st</sup> century investigators in the United States, Australia and in Europe started to conduct risk assessments that clearly involved multiple stressors at the watershed (catchment scale). As of the spring of 2012 these types of assessments can be found examining landscapes in Australia, China, South Africa, United States, and Chile. Some of these assessments use the relative risk model; some Bayesian networks of their own design and others are adapting the relative risk model to a Bayesian network format. In other words there has become an international effort to examine the risks due to multiple stressors on multiple endpoints at very large scales. The stressors being examined are also inclusive. Chemicals of various classes can be included, but non-indigenous species, habitat alteration, and changes in climate are incorporated into the quantitative risk assessment. In many parts of the world regional risk assessment is regarded as a planning tool for the long-term management of important resources. It is also possible to use risk assessment to calculate management scenarios that result in low-risk outcomes to the various endpoints. Recently, application of regional risk assessment has been extended by efforts of the US Forest Service to forest management and to non-indigenous species. My mid 21<sup>st</sup> century the type of risk assessment outlined here will be integrated into a broad environmental assessment, restoration and management scheme incorporating all potential sources, stressors, environments and ecological services. The limitations appear to regulatory and cultural, not that the underpinnings are already there.

**363 Regional Risk Assessment of the Puyallup River Watershed and Low Impact Development (LID) to Meet Management Goals** E.E. Hines, Western Washington University / Institute of Environmental Toxicology, Huxley College of the Environment; S. Edwards, Western Washington University / Institute of Environmental Toxicology; W.G. Landis, Western Washington University, Institute of Environmental Toxicology. Relative risk assessment is a tool to calculate the likelihood of effects to endpoints when multiple stressors occur in complex ecological systems. Here we use Bayesian network (BN) models derived from the relative risk assessment framework. We created a model that can evaluate the risk Low Impact Development is able to reduce in the Puyallup River Watershed for ecological endpoints. The BN structure comprises of three tiers of nodes: landscape stressors, habitats, and ecological endpoints of interest to local managers. Each tier links nodes to lower-tiered nodes when spatial and ecological relationships exist. Four potential discrete states exist for all parameters: zero, low, medium, and high with numeric scores of 0, 2, 4, and 6 respectively. As a first step in model creation, conceptual models show causal pathways between stressors and endpoints. A conceptual model for the endpoint of prespawn mortality in coho salmon (*Oncorhynchus kisutch*) has been created. In the next step, a BN structure has been created for this endpoint, demonstrating causal pathways between the endpoint and the stressors pesticides, polycyclic aromatic hydrocarbons (PAHs), copper, and ecological modifications. The adaptability of using BNs for a relative risk assessment provides opportunity for the model created to be adapted for other watersheds in the Puget Sound region with the same restoration goals. If our model finds Low Impact Development is able to reduce risk, the goal of this model will be to create a relative risk model that may be used in future watershed restoration to determine what types and where Low Impact Development should be implemented.

**365 Accurate bio-activity and toxicity prediction technology based on three-dimensional docking to pocket ensembles and ligand fields** R. Abagyan, University of California, San Diego / Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California San Diego; F. McRobb, Y. Chen, I. Kufareva, University of California, San Diego; M. Totrov, Molsoft LLC. Recognizing a possible endocrine disruptor or a toxic compound among tens of thousands of man-made compounds already present in the environment is vital. Alerting about potential dangers long before a new chemical is developed or released, ideally even before it is synthesized, is an even more important task. Until recently most of the computational models for predicting endocrine disruption were based on quantitative structure-activity relationship models in which a model is 'trained' on a set of chemicals with a certain activity. However, rapid progress in molecular biology, physiology and toxicology allows us to relate many of the effects to direct interactions of compounds with the ligand binding sites of certain receptors, such as nuclear receptors, G-protein coupled receptors, kinases etc. Here we present two three dimensional docking-based technologies, along with the benchmarking, validation and application, which achieve high accuracy of activity prediction without (or with substantially reduced) dependence on "training" on large collections of active chemicals. These unbiased docking based models of several nuclear receptors, including the estrogen receptor, can be now expanded to a sizeable fraction of the comprehensive collection of 3D pockets and co-crystallized ligands, a.k.a. the Pocketome.

**366 Design Guidelines for Minimal Aquatic Toxicity Based on Experimental Spectroscopic Characterization** A. Voutchkova-Kostal, Yale University / Center for Green Chemistry and Green Engineering, George Washington University, George Washington University / Chemistry; N. An, George Washington University / Chemistry. In light of the increasing number of new commercial chemicals that are likely to exert ecotoxicity, the need to *rationally* design chemicals with minimal biological activity is clear. Tools and guidelines must be developed that allow molecular designers to incorporate considerations of minimizing toxicity during the design and synthesis stages. These strategies must be easy to implement alongside traditional chemical synthesis and characterization. The interpretation of chemical characterization data, such as NMR, IR and UV-visible spectroscopy, is second nature to chemists. If such data could be used to inform the likely toxicological hazard of chemicals, the approach to chemical design for minimal hazard could be transformed. Previously we have shown that HOMO-LUMO energy gaps and octanol/water partition coefficients are directly informative of acute and chronic aquatic toxicity. We now show that NMR and UV-visible data can be used in place of calculated properties

to identify chemicals that exhibit high acute and chronic aquatic toxicity. Aquatic toxicity data for *P. promelas*, *D. magna*, *O. latipes* and *P. subcapitata* (72-hr LC50 and NOEC values) were obtained from the U.S. E.P.A. and the Japan Ministry databases, while experimental spectroscopic data were obtained from the NIST database or predicted. We show that although this goal is tremendously challenging, it is feasible and worthwhile of future research efforts.

**367 Designing Benign Chemistries** J. Zimmerman, Yale University; P. Anastas, Yale University / Center for Green Chemistry and Green Engineering. The potential threat that commercial chemicals with unintended biological activity can pose to our society and the environment has become increasingly clear in recent years. While chemists and engineers have developed considerable expertise in designing chemicals to have specific industrial or biological functions, thus far little or no consideration has been given to minimizing the undesired and adverse human health and environmental impacts of commercial chemicals. This work will present strategies and tools to address this critical gap by systematically developing an anticipatory, cohesive molecular strategy for hazard reduction that can readily be 1) implemented in the design stages of a commercial chemical, 2) used by regulatory bodies to evaluate new and existing chemicals to inform design-making on potential hazards, and 3) utilized as the basis for engaging, effective educational materials that ensure molecular designers consider hazard reduction as well as performance criteria. Work has focused on linking physicochemical properties of chemicals to acute ecotoxicological effects. This effort will expand this methodology to extrapolate findings and approaches to broader hazard concerns and endpoints to potentially include chronic and human health endpoints as well as global and physical hazards.

**368 New Computational Toxicology Resources for Green Chemistry** R. Boethling, US Environmental Protection Agency / Office of Pollution Prevention and Toxics; C. Baier-Anderson, E. Sommer, US Environmental Protection Agency; R. Judson, US Environmental Protection Agency / Office of Research and Development; D. Reif, I. Shah, US Environmental Protection Agency; P. Howard, J. Adams, W. Meylan, SRC Inc.. Green chemistry encourages the design or selection of chemicals and chemical processes that reduce or eliminate negative human health and environmental impacts. Principle 4 of the green chemistry principles, "Design safer chemicals," stresses preserving useful function while minimizing toxicity, persistence or other undesirable effects. A principal impediment to its implementation has been the difficulty of finding and retrieving the toxicology, environmental fate and chemical property data required for a holistic perspective of intrinsic hazard. In its Aggregated Computational Toxicology Resource (ACToR), EPA has compiled data from hundreds of sources. ACToR largely solves problems resulting from toxicity data residing in a wide variety of specialized databases, different and often incompatible formats, and different locations. ACToR is being expanded to include data on environmental fate and persistence of chemicals by including the specialized sources and functionality of EPA/SRC's FatePointer tool. In addition to resources already available in ACToR (e.g. ToxCast), users will have easy access to fate data, enabling inclusion of these data alongside toxicity endpoints in alternatives assessment and chemical design. EPA also plans to develop UNIX and web-based versions of EPI Suite™, a tool that predicts chemical properties and fate from chemical structure. The web-based version of EPI Suite™ will be incorporated into customized decision-support tools to enable the integration of chemical property and fate predictions with other types of data and models. Decision-support tools under development include "dashboards", which are tool-based websites tailored to specific subjects or clients; e.g., a web page that allows convenient access to, and application of, the varied resources needed for informed substitution in green chemistry. To illustrate application in chemical or product design, data are retrieved for a series of synthetic musk fragrances, and used to compare alternative structures on the basis of both toxicological and environmental endpoints.

**369 Probabilistic Assessment of Sustainable Molecular Design Guidelines: Exploring Chemical Outliers and Chemical Classes** K.A. Connors, Baylor University / Institute of Biomedical Studies, Baylor University / Department of Environmental Science; A. Voutchkova-Kostal, Yale University / Center for Green Chemistry and Green Engineering, George Washington University, George Washington University / Chemistry; P. Anastas, J.B. Zimmermen, Yale University; B.W. Brooks, Baylor University

/ Environmental Science and Biomedical Studies, Baylor University / Department of Environmental Science. One of the Twelve Principles of Green Chemistry emphasizes the need to synthesize safer chemicals. These chemicals should be capable of performing their desired function but would be designed to elicit minimal toxicity. In support of this effort, two recent studies have explored the relationships between chemical properties and acute or chronic toxicity as measured through standardized OECD and EPA protocols. Mechanistically-rationalized guidelines were derived resulting in two practical design guidelines: acute and chronic toxicity could be minimized if compounds had an octanol-water partition coefficient (log P) below 2 and highest occupied molecular orbital (HOMO)-lowest unoccupied molecular orbital (LUMO) gap (eV) greater than 9 eV. We employed a probabilistic hazard assessment approach to examine the likelihood of encountering industrial chemicals within regulatory toxicity categories (e.g., high, moderate, low toxicity). If these two design guidelines are employed, the present study estimates that acute toxicity to the fathead minnow model could be reduced for over 10% for industrial chemicals classified with 'High' acute toxicity. We further examined chemicals, modes of action and chemical classes for which these property guidelines did not reduce aquatic toxicity. For example, in the absence of chemical design guidelines, our model predicts that 27.6% of chemicals acting through an electrophile reactivity mode of action in the fathead minnow model would be classified as 'High' concern for acute toxicity. When both guidelines are obeyed, only 14.5% of chemicals with an electrophilic MOA would be classified at this level. Ongoing efforts are developing additional design guidelines for chemicals remaining in 'High' toxicity categories.

**370 Quantum-Mechanical Approach to the Prediction of Olefin and Epoxide Mutagenicity** J. Kostal, Sustainability A to Z; A. Voutchkova-Kostal, Yale University / Center for Green Chemistry and Green Engineering, George Washington University, George Washington University / Chemistry; J. Zimmerman, P. Anastas, Yale University. Computational modeling of molecular interactions has found extensive applications in drug discovery; however, its application to the rational design of minimally toxic commercial chemicals has not yet been explored. Present study demonstrates the feasibility of developing computer-aided predictive *in silico* methods for prioritizing chemicals of high concern for human toxicity on the example of epoxide-opening reactions. Epoxides are known for mammalian mutagenicity and carcinogenicity; these toxic endpoints have been attributed to the ability of epoxides to covalently bind to DNA via ring-opening reactions. Activation barriers ( $\Delta G^\ddagger$ ) and free energies of reaction ( $\Delta G_{\text{rxn}}$ ) for the  $S_N2$  ring openings of 13 mono and disubstituted epoxides were computed at the MP2/6-31+G(d,p) levels of theory in the gas phase and in aqueous solution. This study finds an aqueous-solution  $\Delta G_{\text{rxn}}$  threshold value of -15 kcal/mol can be used to discern mutagenic/carcinogenic epoxides ( $\Delta G_{\text{rxn}} < -15$  kcal/mol) from non-mutagens/non-carcinogens ( $\Delta G_{\text{rxn}} > -15$  kcal/mol). The computationally modeled reactivity of epoxides is thus proposed as a viable *in silico* screen for potentially toxic epoxides.

**371 Understanding the Impact of SWNT Aggregation on Toxicity to Inform Greener Nano Design** L. Pasquini, S. Hashmi, M. Elimelech, J. Zimmerman, Yale University. Functionalized SWNTs (fSWNTs) are a unique class of emerging nanomaterials that offer simultaneous enhancement of SWNT applications and reduction of their negative human health and environmental implications. Here, the percent cell viability loss of *Escherichia coli* K12 resulting from exposure to nine fSWNTs is explored. Three of the fSWNTs exhibit statistically significant decrease in percent cell viability loss. Various characterization techniques were employed to characterize the fSWNTs and identify the physicochemical properties associated with the observed trend in bacterial cytotoxicity. Light scattering results, both static (SLS) and dynamic (DLS), elucidated aggregate morphology and provided the distribution of diffusion time of fSWNT aggregates under experimental conditions. Individually, DLS and SLS do not explain the observed trend in cell viability loss, but combined it becomes clear that compact monodispersed fSWNT aggregates result in reduced cytotoxicity. This is the first study that takes a systematic approach to determine fSWNT physicochemical properties that result in the observed trend in cytotoxic behavior fSWNTs. The targeting of specific properties is intended for future design and manufacture of safer SWNTs.

**372 Greener Organocatalysis: Toxicity and Biodegradation studies in tandem with Green Chemistry Metrics analysis** [N. Gathergood](#), Dublin

City University / School of Chemical Sciences; S. Connon, University of Dublin, Trinity College / School of Chemistry; R. Gore, Dublin City University / School of Chemical Sciences; L. Myles, University of Dublin, Trinity College / School of Chemistry; T. Garcia, IQAC-CSIC / Department of Surfactant Technology. The research project combines two fields of green chemistry, ionic liquids and catalysis. Our study is directed towards ionic liquids which can also catalyse reactions. This overlap between organocatalysis and ionic liquid research enables us to design low toxicity and potentially biodegradable catalysts based on the extensive biological screening data of ionic liquids. Assessment of the performance of a new catalyst, in tandem with the (eco)toxicity screening and biodegradation testing allows the chemist to develop green synthetic methods. We have designed a library of aprotic ionic liquids which can act as Brønsted acid catalysts with low antibacterial and antifungal activity. Preliminary studies of the toxicity of all the ionic liquids were performed to establish the influence of the ester or amide group in the cation. Antifungal and antibacterial toxicity studies demonstrated that the ionic liquids did not inhibit the growth of any organism screened at concentrations of 2.0 mM. Biodegradation of all novel ionic liquids was investigated in "CO<sub>2</sub> Headspace" test (ISO 14593). Our latest studies (including Green Chemistry metrics feedback) investigate the effect of substitution on the imidazolium ring on biodegradation and activity of the catalyst. Acetalisation and thioacetalisation reactions of a variety of aldehydes have been studied at room temperature and low catalyst loadings (0.05 mol%). The study demonstrated the excellent catalytic activity of these aprotic molecules as active Brønsted acids in the presence of protic media. The most active catalyst has been recycled 15 times without any loss of catalytic activity.



**373 CECs – Why the Big Fuss?** S. Aminzadeh, California Coastkeeper Alliance. Contaminants of emerging concern (CECs) are increasingly detected at low levels in California's waterbodies. Some CECs are similar to conventional toxic pollutants in that they are associated with industrial releases, whereas many others are used by the general public in pharmaceuticals and personal care products. CECs are released directly to the environment after passing through wastewater treatment processes, which are typically not designed to remove CECs from the effluent. These contaminants are relatively unmonitored and found to occur at trace levels in wastewater discharges, ambient receiving waters, and drinking water supplies. Many CECs are so new that standardized measurement methods and toxicological data for interpreting their potential human or ecosystem health effects are unavailable. This lack of basic information and technology to efficiently measure CECs hampers California's ability to assess their potential risks and develop regulatory protocols. The discharge of CECs to California's receiving waters occurs on a daily basis due to recycled water. As recycled water becomes an increasingly important part of California's water supply portfolio, the State faces the challenge of monitoring and regulating the discharge of CECs into surface and groundwater. Many streams in southern California are effluent-dominated streams with 80-95% of dry weather flows coming from recycled water discharges, and many northern California streams receiving recycled water effluent interact regularly and closely with groundwater. As such, the importance of monitoring for CECs is absolutely critical to identify risks posed to public health and aquatic life. Some CECs have been shown to cause endocrine disruption after being released to the environment, as they interfere with the action of reproductive hormones such as estrogen and testosterone. CEC standards are critical to build public confidence in the use of recycled water; and the absence of monitoring data for CECs is an impediment to the promotion of recycled water as a sustainable water supply for California.

**374 Regulatory Aspects of Managing CECs in California Waters** J. Bishop, California Water Resources Control Board. California recycles approximately 650,000 acre-feet of water per year, but has identified the potential to reuse an additional 1.5 million acre-feet in the future. To encourage expanded reuse in a state that experiences periodic water shortages, the California State Water Resources Control Board (SWRCB) adopted a Recycled Water Policy in 2009 intended to provide permitting clarity for recycled water projects. One challenge in developing that policy was how to address new classes of chemicals, such as pharmaceuticals, current use pesticides, and industrial chemicals, collectively referred to as chemicals of emerging concern (CECs). Many CECs are potentially present not only in recycled water, but also in ambient waters that receive discharge of treated municipal wastewater effluent and stormwater runoff. After discharge to receiving waters, CECs may have direct impacts to aquatic life, or may associate with particles, accumulate in sediments and work their way up the food chain to higher biota, including humans. The mission of the SWRCB is to protect beneficial uses of water resources in California through promulgation of water quality standards and monitoring requirements for permitted discharges, ground and surface waters. To address the different challenges that CECs present, the Water Board also supports research and technology development that facilitates science-based decision and policy making. One such example is the development of bioanalytical screening tools, a means for detection of many known and unknown chemicals based on their mode of biological action, thus de-emphasizing the need to develop chemical specific analytical methods for the next generation of CECs.

**375 Monitoring CECs in San Francisco Bay** M. Sedlak, D. Yee, J. Davis, San Francisco Estuary Institute. The Regional Monitoring Program for Water Quality in the San Francisco Estuary (RMP) has been investigating contaminants of emerging concern (CECs) since 2001 and developed a formal workgroup to address the issue in 2006. The RMP Emerging Contaminants Workgroup (ECWG) includes representatives from RMP stakeholder groups (wastewater, stormwater, and industrial dischargers, dredgers, and regulatory agencies) and an advisory panel of expert researchers that work together to address the workgroup's guiding management question – Which CECs have the potential to adversely impact beneficial uses in San Francisco Bay? The overarching goal of the ECWG is to develop cost-effective strategies to identify and monitor CECs so that potentially problematic chemicals can be identified as early as possible and thus minimize impacts to the Estuary. The ECWG works toward this goal by evaluating available information on chemical occurrence, fate,

toxicity, volume use, and potential sources, and then recommends CECs for investigation in special studies. Each year the highest priority studies are conducted, and the results guide whether or not these CECs are added to routine monitoring by the RMP. Using this process, the RMP has generated one of the most comprehensive datasets for CECs in an aquatic ecosystem. CECs investigated to date include perfluorinated compounds, alkylphenols, more than 100 pharmaceuticals and personal care product ingredients, and a variety of flame retardants including polybrominated diphenyl ethers and their replacements. This presentation will summarize results from the CEC studies conducted to date and highlight the ECWG approach to prioritizing CECs for monitoring.

**376 CA Science Advisory Panel recommendations for CEC monitoring in recycled water** P. Anderson, Ohio EPA; N.D. Denslow, University of Florida / Department of Physiological Sciences and Center for Environmental and Human Toxicology; J. Drewes, Colorado School of Mines / Environmental Science and Engineering; K.A. Maruya, SCCWRP, Southern California Coastal Water Research Project; A. Olivieri, EOA, Inc.; D. Schlenk, University of California-Riverside / Department of Environmental Sciences; G.I. Scott, National Ocean Services / Center for Coastal Environ. Health & Biomolecular; S. Snyder, University of Arizona / Chemical and Environmental Engineering. The paucity of information on the occurrence and potential impacts of chemicals of emerging concern (CECs) in natural and treated waters currently limits our ability to define and, thus, manage risks associated with CECs. In response, the State of California tasked Southern California Coastal Water Research Project (SCCWRP) to convene an expert panel to provide recommendations for effective monitoring of CECs in recycled water. Through an open and transparent process, SCCWRP convened a panel of six national experts in the fields of chemistry, biochemistry, toxicology, epidemiology, risk assessment and engineering, with more than 100 years of combined experience investigating CEC issues. The Panel was asked to address five charge questions. They held several meetings during which they considered a wide range of CECs and identified the scenarios most likely to be associated with exposure to CECs. The Panel created a framework to prioritize CECs based on occurrence and toxicological relevance, applied this framework using statewide data and provided recommendations for monitoring of high priority CECs and treatment performance indicators and surrogates. In addition, the Panel identified data gaps, research needs and programmatic issues that would result in future improvements to the framework and increase the overall relevance and effectiveness of CEC monitoring in recycled water.

**377 Monitoring Strategies for Chemicals of Emerging Concern (CECs) in California's Aquatic Ecosystems Recommendations of a Science Advisory Panel** D. Schlenk, University of California-Riverside / Department of Environmental Sciences; K.A. Maruya, SCCWRP, Southern California Coastal Water Research Project; P. Anderson, Ohio EPA; N.D. Denslow, University of Florida / Department of Physiological Sciences and Center for Environmental and Human Toxicology; A. Olivieri, EOA Inc.; J. Drewes, Colorado School of Mines / Environmental Science and Engineering; G. Scott, NOAA; S. Snyder, University of Arizona / Chemical and Environmental Engineering. Contaminants of emerging concern (CECs) represent a challenging problem for regulators to address, owing to limited scientific knowledge about their sources, fates, and effects. The State of California convened a panel of experts to provide recommendations on how current knowledge of CECs should influence their regulatory activities, beginning with recycled water applications, and with support from the David and Lucile Packard Foundation, continuing with ambient waters receiving treated wastewater effluent and stormwater discharge. The Panel delivered a risk-based framework and monitoring approach which included a list of priority CECs, appropriate methods and interpretive guidelines for monitoring data. To make future monitoring more relevant and efficient, the Panel recommended the development of bioanalytical tools that integrate the response of high priority CECs.

**378 Developing bioanalytical techniques to address water quality** N.D. Denslow, University of Florida / Department of Physiological Sciences and Center for Environmental and Human Toxicology; P. Anderson, Ohio EPA; J. Drewes, Colorado School of Mines / Environmental Science and Engineering; A. Olivieri, EOA, Inc.; D. Schlenk, University of California-Riverside / Department of Environmental Sciences; G.I. Scott, National Ocean Services / Center for Coastal Environ. Health & Biomolecular; S.

Snyder, University of Arizona / Chemical and Environmental Engineering; S. Westerheide, University of South Florida; K.A. Maruya, Southern California Coastal Water Research Project, SCCWRP. Standard methods to evaluate occurrence of chemicals in surface waters or reused water currently involve a chemical-by-chemical approach using mass spectrometry. However most of the analytical methods that exist in the commercial sector are primarily for select groups of chemicals that have previously been identified as chemicals that should be regulated. This includes high volume pollutants and legacy pesticides. In the past several decades, it has become apparent that additional chemicals, typically referred to as chemicals of emerging concern (CECs), including many pharmaceuticals and personal care products may also appear in surface waters that receive treated sewage influents; however, analytical methods for CECs are scant and not standardized. While many of the CECs can be predicted to be in the waters based on their physical-chemical properties and their volume of production and use, there are other CECs which may be present but which we cannot predict and consequently for which we do not have any analytical methods. These are the "unknown unknowns", i.e., chemicals which we do not anticipate being in the water and for which we have no analytical methods. This group may include additional pharmaceuticals, by-products of disinfection, or novel replacement pesticides, among others. It is possible to measure occurrence of these chemicals by their molecular activities, using high throughput bioanalytical assays. Of special concern are chemicals that may be additive (or synergistic) in their molecular activities and which may activate adverse outcome pathways, leading organisms towards decreased growth, decreased reproduction, increased susceptibility to disease or even death. We are using these high throughput assays to determine chemical equivalencies (for example, total estrogen equivalency) and comparing the bioanalytical measurements with traditional mass spectrometry methods.

**379 The Role of Non-Targeted Analyses in the Evaluation of CECs** S. Snyder, University of Arizona / Chemical and Environmental Engineering; S. Merel, A. Jia, University of Arizona; N. Dodder, Southern California Coastal Water Research Project; K.A. Maruya, SCCWRP; Southern California Coastal Water Research Project. The vast majority of analytical techniques applied in the environment are designed to identify and quantify specific targeted contaminants. However, targeted analytes provides only limited resolution regarding the actual occurrence of environmental contaminants. Moreover, many targeted analytes are transformed by natural and/or engineered processes to generate transformation products of unknown structure and toxicity. The use of bioassays provides tremendous advantage in screening for wide ranges of contaminants with specific biological activity. Yet, bioassays are generally unable to distinguish the specific contaminants responsible for observed bioactivity. Non-targeted analytical techniques are critical in identification and new environmental contaminants, transformation products, and natural products in complex environmental matrices. For instance, time of flight mass spectrometry is capable of provide high mass accuracy valuable in calculation of molecular formula. Sample preparation and separation technologies including on-line SPE and GCxGC/LCxLC techniques are robust technologies for identifying non-targeted contaminants. Beyond analytical instruments, advanced software platforms are capable of complex statistical analyses which can differentiate unique peaks within multiple matrices. Lastly, coupling in vitro bioassay techniques with non-targeted analyses provides for a bio-directed analytical approach that has been successfully applied to estrogenic and genotoxic environmental contaminants. We will provide specific examples of non-targeted analyses successfully employed for identification of environmental contaminants in fish tissues, water, and sediments.

**380 Development of Standardized Methods for Assessing Antibiotic Resistance Risks** G.I. Scott, National Ocean Services / Center for Coastal Environ. Health & Biomolecular; M. Fulton, J. Moore, NOAA/NCCOS; S. Norman, M. Uyaguari, University of South Carolina; D. Schlenck, University of California; N.D. Denslow, University of Florida; P. Anderson, ARCADIS and Boston University; J. Drewes, Colorado School of Mines; A. Olivieri, EOA, Inc.; S. Snyder, University of Arizona; K. Maruya, Southern California Coastal Water Research Program. Chemicals of Emerging Concern (CECs) may include a wide variety of chemicals such as flame retardants, contemporary use pesticides, newly developed commercial products (e.g. nanomaterials) and pharmaceutical/personal care products for which there are often incomplete ecotoxicity data. Antibiotics are CECs that have received significant attention due to their potential to

cause development of bacterial resistance at very low concentrations and/or development of resistance genes that may be exchanged (e.g. plasmids) among bacteria. Between 100 to 200 million kg of antibiotics are consumed annually worldwide, while in the US, more than 22 million kg of antibiotics are produced annually for animal (40%) and human (60%) use. Most antibiotics are poorly absorbed, resulting in 25 – 75% of the consumed product being released unaltered in feces or urine, which may be discharged into sewerage treatment plants (STPs). STPs concentrate and treat human waste, ultimately releasing treated wastewater into local environments. While STPs efficiently remove most biosolids, many antibiotics and antibiotic resistant bacteria often survive the treatment process and may be discharged into the environment. High levels of antibiotic resistance have been reported among bacteria in water, sediments and biota in aquatic ecosystems as a result. Antibiotics may adversely affect bacteria resulting in death at high therapeutic doses [Minimum Inhibitory Concentrations (MICs)] while at lower doses bacteria may survive and adapt to exposure by mutations which may result in development of antibiotic resistance (ABR). MICs are generally the antibiotic concentrations that cause bacterial death and are routinely reported for every antibiotic. Published MIC data for *E. coli* or other commonly used bacteria in water quality monitoring or research were used to determine toxicity thresholds for antibiotics that have been measured in monitoring studies conducted throughout the US. A process was developed to assess the potential for development of ABR which compared MICs for antibiotics under a variety of exposure conditions. The ratio of Standard and Resistant MICs was then used as an estimate of the potential for enhancement of ABR within the environment and safety factors were applied to deal with uncertainty. Methods for assessing the potential for developing ABR from low levels exposure of antibiotics versus exchange of genes from plasmids among resistant bacteria will be discussed.

**381 Algal blooms in European lakes: overview of assessment methods and a modelling case study** J. Moe, Norwegian Institute for Water Research (NIVA) / Climate and environmental modelling, Norwegian Institute for Water Research / Climate and environmental modelling, Norwegian Institute for Water Research / Section for Freshwater Biodiversity, Norwegian Institute for Water Research (NIVA) / Section for Climate and Environmental Modelling; D.N. Barton, Norwegian Institute for Nature Research (NINA); B. Skjelbred, A.L. Solheim, Norwegian Institute for Water Research (NIVA). Eutrophication (excessive nutrient enrichment) is one of the greatest environmental problems for inland waters of Europe, especially in agricultural areas. High nutrient concentrations (phosphorus and nitrogen) in lakes promote algal blooms, and thereby increase the risk of toxin-producing cyanobacteria. In addition, climatic change such as increased temperature tends to favour growth of harmful cyanobacteria relative to other algae. Environmental legislation in the European Union (The Water Framework Directive) requires that the frequency and intensity of algal blooms in lakes are only "slightly higher" than in comparable undisturbed lakes. A research challenge across Europe has been to quantify an acceptable "slight increase" of blooms and other metrics describing of the phytoplankton community, and to relate this management goal to nutrient pressures in different geographic regions and for different lake types. This presentation will give an overview of current assessment methods for cyanobacteria and other phytoplankton metrics for European lakes. A case study from the eutrophic Lake Vansjø in Norway will also be presented. This lake has experienced cyanobacteria blooms for decades, in spite of considerable nutrient abatement measures. The blooms occasionally cause production of toxins (e.g. microcystin) which are harmful to people, and result in restrictions on recreational use of the lake. We use a Bayesian network modelling approach to integrate information on climatic variables, nutrient concentrations, algal biomass etc., and simulate impacts of alternative abatement measures on levels of cyanobacteria under different climatic conditions.

**382 Harmful Algal Bloom Toxins in Inland Waters: Perspectives on Assessment and Management of these Environmental Contaminants of Emerging Concern** B.W. Brooks, Baylor University / Environmental Science and Biomedical Studies, Baylor University / Department of Environmental Science. Environmental science and management continuously faces emerging challenges associated with contaminants for which limited information is available for their fate and effects. Regulatory programs have developed approaches to characterize risks from chemicals and chemical management programs; however, such approaches are not as widely defined or employed, however, for harmful algal blooms (HABs), whose increase

in frequency and magnitude over the past few decades has occurred at the global scale. Factors leading to development of HABs vary, but climate change and urbanization are common influences on such increases of HABs. Though much attention has been given to coastal HABs, inland waters often experience HABs, which can be exacerbated by anthropogenic activities. For example, harmful blooms of cyanobacteria receive unprecedented attention due to impacts on fisheries, linkages to terrestrial poisonings, and public health concerns associated with drinking water contamination. A number of *Prymnesium parvum* (golden algae), an invasive haptophyte historically observed in estuarine and coastal habitats, have increasingly produced devastating fish kills in inland waters of the United States. Managers no longer stock sport fish in some impoundments due to repeated, fish-killing *P. parvum* blooms, and seasonal *P. parvum* blooms in water supply reservoirs have resulted in cessation of municipal drinking water withdrawals. This presentation critically examines HAB toxins as contaminants of emerging concern, and provides specific recommendations within the framework of water quality assessment and management as mandated by the Clean Water Act.

**383 Influence of Spatiotemporal pH Variability on Site-specific Water Quality Assessments** W.C. Scott, Baylor University / The Institute of Ecological, Earth, and Environmental Sciences; K.N. Prosser, Baylor University; B.W. Brooks, Baylor University / Environmental Science and Biomedical Studies, Baylor University / Department of Environmental Science. Water chemistry gradients can develop along longitudinal and vertical axes in aquatic systems due to natural and anthropogenic activities. Gradients of pH, temperature and salinity, for example, can form longitudinally and vertically in both inland and coastal systems. In particular, production: respiration dynamics and the formation of thermal stratification can cause pH levels to vary through a water column. Such changes in pH can influence the ionization state and bioavailability of ionizable compounds, resulting in differential spatiotemporal bioaccumulation and toxicity of these contaminants. The objective of this study was to develop an understanding of spatiotemporal pH variability in surface waters to support future hazard assessments of contaminants exhibiting pH-dependent toxicity. We selected three coves in Lake Granbury, a continuous level impoundment of the Brazos River located in central Texas, USA, for initial studies during Winter and Spring 2012. Our observations revealed that the mean change in pH through a water column more than doubled in all three coves sampled from February to April, and in some cases varied by 2 orders of magnitude. Furthermore, the magnitude of vertical pH gradients appeared to decrease with distance upstream from the main channel as depth decreased. Harmful blooms of *Prymnesium parvum* blooms are more toxic at higher pH and primarily occur in the winter and early spring months in Texas. February studies on Lake Granbury revealed uniformly high pH throughout the water column, whereas April studies showed deep zones of water with lower pH that could potentially serve as a refuge from toxicity. Criterion Maximum Concentrations (CMC) and Site Specific Potency Ratios of ammonia and pentachlorophenol varied significantly with depth between February and April across all three coves. Mean surface and bottom CMC for ammonia in the three coves were predicted to be 15.50 and 17.77 TN/L, respectively, for February, and 4.17 and 8.54 TN/L for April. Likewise, mean surface and bottom CMC for pentachlorophenol for the three coves in February were predicted to be 24.56 and 22.53 ug/L, respectively, and 30.73 and 19.93 ug/L for April. Ongoing research of Lake Granbury will extend to summer and fall sampling events, and further expand this study to include rivers flowing to Gulf Coast estuaries to better understand temporal patterns of pH gradients and how they may impact the hazards of ionizable contaminants.

**385 Neurotoxic effects of saxitoxins in freshwater fish: in vivo and in vitro studies** C.A. da Silva, Federal University of Paraná / Ecology and Conservation Post-graduate Program; E.T. Oba, Federal University of Paraná / Department of Pharmacology; E.C. Morais, M.D. Moura Costa, Federal University of Paraná / Department of Basic Pathology; J.C. Ribas, I.C. Guiloski, Federal University of Paraná / Department of Pharmacology; W.A. Ramsdorf, Federal University of Paraná / Department of Genetics; S.M. Zanata, Federal University of Paraná / Department of Cellular Biology; M.M. Cestari, Federal University of Paraná / Department of Genetics; C.A. Oliveira Ribeiro, Federal University of Paraná / Department of Cellular Biology; V.F. Magalhaes, Federal University of Rio de Janeiro / Carlos Chagas Filho Institute; H. Silva de Assis, Federal University of Paraná.

Cyanobacteria blooms saxitoxin (STX) producers such as *Cylindrospermopsis raciborskii*, has become a serious environmental concern, especially in southern Brazil, where this species has already been dominant in public water supply reservoir. STX is a naturally produced neurotoxin that blockage the sodium channel in the neurons cells leading animals to death. However, the bioaccumulation in trophic chain and their neurotoxicological effects in fish are not acknowledged completely. To better understand the potential of bioaccumulation of STX, the freshwater fish *Hoplias malabaricus* was submitted to a trophic bioassay (0.08 µg/100 g of STX equivalents) using individuals of *Astyanax sp.* as vehicle during 20 days with feeding every 5 days. The biomarkers as activities of acetylcholinesterase (AChE), superoxide dismutase (SOD), catalase (CAT), glutathione S-transferase (GST) and glutathione peroxidase (GPx), the concentration of glutathione (GSH), lipoperoxidation (LPO), protein carbonylation (PCO), and comet assay were analyzed in the brain. Muscle was collected for STX chemical analysis. To evaluate neurotoxic effect directly in the target cell, a new protocol was established using primary culture of neurons from *H. malabaricus*. The cells were incubated at 24°C, 1.7% CO<sub>2</sub> for 24h and the medium was changed to a new one without and with STX (0.3 µg/L and 3 µg/L) for 24h. Isolated cells were immunocytochemistry marked. Cell viability, oxidative stress, apoptosis and genotoxic effects were analyzed. The results of the bioassay suggested an oxidative stress and genotoxicity. However, it was not observed STX contents in muscle samples. The in vitro protocol showed that a culture medium enriched with fibroblast growth factor and nutritional supplements such as B27, and the use of papain, as well as substrate coating appropriate as poly-L-Lysine and segregation gradients cells, allowed a cellular culture with good cell viability. After the neurons exposure to STX cytotoxicity, oxidative stress, and genotoxicity were observed. It was not noticed apoptosis in the exposed group. Although STX effects are reported as reversible, the time exposure and concentration of STX suggested cellular damage which can lead to neuropathology. In addition, the establishment of neurons culture primary protocol enables new applications in fish ecotoxicology in the assessment of neurotoxic substances in the target cells.

**386 State of the Art and Research Needs on the Management of Harmful Cyanobacterial Bloom** S. Merel, The University of Arizona / Chemical and Environmental Engineering; D. Walker, The University of Arizona / Environmental Research Laboratory; R. Chicana, Cesar Vallejo University / School of International Business; S. Snyder, The University of Arizona; O. Thomas, French School of Public Health / Environment and Health Research Laboratory. Cyanobacteria are ubiquitous microorganisms considered as important contributors to the formation of Earth's atmosphere and nitrogen fixation. However, they are also frequently associated with toxic blooms. Indeed, the wide range of hepatotoxins, neurotoxins and dermatotoxins synthesized by these bacteria is a growing environmental and public health concern. This study provides a state of the art on the management of harmful cyanobacterial blooms in surface and drinking water, including economic impacts and research needs. Cyanobacterial blooms usually occur according to a combination of environmental factors e.g., nutrient concentration, water temperature, light intensity, salinity, water movement, stagnation and residence time, as well as several other variables. These environmental variables, in turn, have promoted the evolution and biosynthesis of strain-specific, gene-controlled metabolites (cyanotoxins) that are often harmful to aquatic and terrestrial life, including humans. Cyanotoxins are primarily produced intracellularly during the exponential growth phase. Release of toxins into water can occur during cell death or senescence but can also be due to evolutionary-derived or environmentally-mediated circumstances such as allelopathy or relatively sudden nutrient limitation. Consequently, when cyanobacterial blooms occur in drinking water resources, treatment has to remove both cyanobacteria (avoiding cell lysis and subsequent toxin release) and aqueous cyanotoxins previously released. Cells are usually removed with limited lysis by physical processes such as clarification or membrane filtration. However, aqueous toxins are usually removed by both physical retention, through adsorption on activated carbon or reverse osmosis, and chemical oxidation, through ozonation or chlorination. While the efficient oxidation of the more common cyanotoxins (microcystin, cylindrospermopsin, anatoxin and saxitoxin) has been extensively reported, the chemical and toxicological characterization of their by-products requires further investigation. In addition, future research should also investigate the removal of poorly considered cyanotoxins (β-methylamino-alanine, lyngbyatoxin or aplysiatoxin) as well as the economic impact of blooms.



**387 The Prevalence of Cyanobacteria and Cyanotoxins in Southern California Waterbodies**

M. Howard, Southern California Coastal Water Research Project; R. Kudela, University of California, Santa Cruz; M. Suttle, E. Stein, Southern California Coastal Water Research Project; L. Busse, San Diego Regional Water Quality Control Board; D. Greenstein, S. Bay, Southern California Coastal Water Research Project; T. Magrann, Saddleback College. Cyanobacterial blooms are a global problem and have been documented throughout California in estuarine, brackish and fresh water habitats. Blooms of cyanobacteria cause a variety of water quality problems such as impaired recreational waterbodies, influenced biological index score, reduced aesthetics, lower dissolved oxygen concentrations, taste and odor problems in drinking water and the production of toxins (cyanotoxins) which have caused wildlife mortality and are associated with liver cancer and tumors in humans. The impact of cyanotoxins can extend well beyond freshwater systems and have created a land-sea connection by causing mortality in California sea otters. Despite the health risks associated with cyanotoxins, little is known about the predominance of cyanotoxins in southern California waterbodies. The prevalence of cyanobacteria and cyanotoxins and the potential environmental drivers of cyanotoxin production were evaluated in a variety of southern California waterbodies using conventional monitoring methods combined with a newly developed monitoring tool, SPATT (Solid Phase Adsorption Toxin Tracking). Cyanotoxins and associated chemical and biological constituents were measured including nutrients, pH, alkalinity, electrical conductivity, total dissolved solids, dissolved oxygen, chlorophyll *a* and algal pigments from over 60 depressional wetlands, lakes, ponds, bays and lagoons in southern California. Laboratory studies were conducted to evaluate the potential effects of cyanotoxins on benthic species used in standard toxicity tests.

**388 Algal toxins and human health: A brief overview and notes on needed research directions**

N. Chernoff, United States Environmental Protection Agency / Toxicology Assessment Division; NHEERL, ORD. Marine and freshwater algae, both algae and cyanobacteria, are known to produce a wide variety of toxins that have caused severe adverse health effects in humans and other vertebrate species. There is general agreement that there is currently an increase in the incidence of hazardous algal blooms (HABs), the periods of occurrence, and their geographical range. The primary reasons for these changes include eutrophication of water bodies, climate change, global spread of individual species, and their intrinsic adaptability. Some toxins are known to be produced by only a small number of organisms while others (e.g. cylindrospermopsin (CYN) and saxitoxin) are generated by multiple species in evolutionarily distant groups. The toxins in HABs may induce general effects (CYN) or may target specific organ systems like the liver (microcystins) or the central nervous system (anatoxin-a, saxitoxin). Poisoning episodes involving severe toxicity and/or lethality have been recorded in humans after exposure to the above toxins as well as many others. Laboratory animal models have been developed for many of the toxins and there is excellent agreement in the types of toxicity observed across mammalian species. Lower vertebrates and invertebrates, however, often show different spectrums of toxicity and may display significant bioaccumulation of toxins. Many fundamental areas of research require additional work. The toxicity of mixtures is a critical data gap because exposures to single toxins rarely, if ever, occur. Reasons for this include the fact that during blooms, other species producing different toxins may be present; many species produce more than one toxin during a bloom; and blooms often occur sequentially in terms of the predominant species, and exposure to liver toxins may, for example, be followed by neurotoxin exposure. Additional underemphasized research areas include mechanism studies, and both developmental toxicology and long-term exposure studies (including carcinogenic potential) in susceptible organisms. The possible role of picoplankton in the production of toxins needs investigation as some types have been shown to produce microcystins. There is a need for a reliable biomarker of exposure in humans. Finally, interdisciplinary work is necessary in comprehensive toxicology studies. Information on bloom taxonomy, conditions and toxin production, and toxin analyses are essential to maximize the relevance of laboratory data to environmental HAB exposures.

**389 Commercial Nanosilver Applications and Their Potential for Environmental Release**

J.A. Steevens, U.S. Army Engineer Research & Development Center / Waterways Experiment Station; A. Kennedy, M. Chappell, F. Hill, A. Poda, D. Johnson, US Army ERDC. Historically silver has found a wide range of applications including its use as an antimicrobial

agent. More recently, nanoscale silver has been explored for use in washing machines, water treatment, catalysis, medical applications, and sensors. The antibiotic applications of nanosilver include use to kill bacteria, fungi, and viruses. The greatest potential for commercialization exists through use of nanosilver (< 50 nm) as a component of self-decontaminating surfaces and fabrics. Many of these antibiotic applications require a composite of NP silver with other metals such as titanium dioxide for water and air purification. The mechanism of action for nanosilver is through silver ion release and surface charge interactions that result in free radical generation and membrane disruption. To understand the environmental releases of nanosilver, the technologies can be evaluated through a life-cycle approach where releases can be examined throughout development, manufacture, use, disposal, and recycling. Because nanosilver is incorporated in a surface, fabric, or composite releases must occur from the technology. The releases can occur through passive release from an unstable matrix or by release through abrasion, dissolution, thermal and chemical decomposition, or biotic transformation. Nanosilver technologies often require direct contact with water (i.e., washing, fabric, water treatment) therefore several investigators have described or quantified releases through wastewater treatment plants. The estimates made to quantitatively predict the quantity of nanoscale materials released to the environment have a high degree of uncertainty and remain very dynamic due to the increasing number of producers, applications, and unknown nature and stability of the NP in the technology. This presentation will provide an introduction to nanoscale silver technologies and describe approaches used to decrease uncertainty in the estimates of environmental release.

**390 Fate and effects of silver nanoparticles in terrestrial environments**

J.M. Unrine, University of Kentucky, University of Kentucky / Department of Plant and Soil Sciences, University of Kentucky / Department of Plant & Soil Sciences, University of Kentucky / Savannah River Ecology Laboratory, University of Kentucky / Department of Plant and Soil Sciences, University of Georgia / Savannah River Ecology Laboratory; P. Bertsch, O. Tsyusko, University of Kentucky; W.A. Shoults-Wilson, Roosevelt University; G.V. Lowry, Carnegie Mellon University; B.P. Colman, E.S. Bernhardt, Duke University. Terrestrial environments are likely to be the ultimate sink for the majority of manufactured silver nanoparticles (AgNPs). As nano-Ag products are used and particles enter wastewater streams, they ultimately partition into sewage sludge, the majority of which is used as a soil amendment in agro-ecosystems. This raises several important questions. First, how are AgNPs transformed during wastewater treatment? How do the speciation and distribution of AgNPs change over time in biosolid amended soils, and how does this affect their bioavailability and toxicity? Finally, can simple laboratory toxicity assays predict ecological consequences in real-world exposures? We have found that AgNPs are extensively transformed during wastewater treatment, with sulfidation playing the most important role. These transformations dramatically alter the behavior of AgNPs in soil pore-water, and decrease the role of the original manufactured coating. For example, for freshly synthesized particles, citrate-coated AgNPs remained stable in soil pore water for up to six months while polyvinylpyrrolidone-coated AgNPs rapidly partitioned into solid phases. However, when pre-incubated with biosolids, the presence of free AgNPs in soil pore water was dramatically reduced for both coatings. Several studies have investigated the toxicity of unsulfidized AgNPs to earthworms in soil. The most sensitive endpoint measured to date was behavioral avoidance, which occurred at AgNP concentrations that were similar to estimates of Ag concentrations already present in some biosolids. Fewer toxicity studies have been conducted using exposures with sulfidized Ag MNMs, and fewer still in natural soils and sediments. Simple laboratory assays indicate that the solubility of sulfidized AgNPs is dramatically decreased as is toxicity. However, in natural soils and sediments Ag<sub>2</sub>S solubility may be enhanced, possibly due to microbial activity or redox coupling with other mineral phases. A recent mesocosm study using AgNPs pre-incubated in sewage sludge indicated that in more realistic, multi-species exposures, unpredicted indirect effects may occur, with effects on plant and microbial communities being detected at µg/kg concentrations in soil. Major efforts are currently underway regarding risks of nano Ag in terrestrial environments that will help to answer these basic questions.

**391 Ecotoxicological Effects of Nanosilver on Soil Microorganisms**

X. Lu, Peking University / Lab for Earth Surface Processes, College of Urban and Environmental Sciences; C. He, F. Gao, Z. Hou, S. Zhang, J. Yang,

Peking University / College of Urban and Environmental Sciences. With the rapid development of nanotechnology, manufactured nanomaterials are increasingly used and may contaminate the soil during the process of their use. Nanosilver is widely used due to its unique biocompatibility and antibacterial properties. The toxic impacts of nanosilver on microbes have been revealed by some researchers, but these researches were mainly based on pure cultures other than microbes in complicated matrix. This study aimed at investigating the ecotoxicological effects of nanosilver on soil microorganisms. Fresh soil from a hillside was collected for the experiment. Nanosilver with particle diameter of 15 nm and purity of 99.99% was purchased from a company. Using self-designed apparatus, the nanosilver was mixed with the soil at concentration of 1 mg nanosilver per gram of fresh soil and then split into 18 Erlenmeyer flasks. Fresh soil without nanosilver was used as control and also split into 18 Erlenmeyer flasks. All the flasks were covered with gauze and incubated in a cool and dark closet. Periodically, distilled water was added to each of the flasks to maintain the soil water content at about 5%. Every four weeks, three flasks of the nanosilver treated soil and three flasks of the control soil were taken to analyze different ecotoxicological parameters including microbial biomass, biochemical processes (respiration and ammonification), activities of enzymes (dehydrogenase and fluorescein diacetate esterase) and community structure. After near five months of experiment, the results showed the activities of both dehydrogenase and fluorescein diacetate esterase were inhibited by nanosilver; respiration of the soil microorganisms was not influenced by the nanosilver, while ammonification was stimulated by the nanosilver in the beginning but inhibited later on; no significant differences in microbial biomass and community structure was observed between the nanosilver treated soil and the control soil. The study results will be discussed in detail in terms of the experiment design, the parameter sensitivities, and the mechanisms involved in the ecotoxicological effects.

**392 Bioaccumulation and Toxicity of Silver Nanoparticles to Benthic Organisms** H. Selck, Roskilde University / Department of Environmental, Social and Spatial Change, Roskilde University / Dept. Environmental, Social and Spatial Change; G. Banta, Roskilde University / Environmental, Social & Spatial Change (ENSAPC); V.E. Forbes, University of Nebraska Lincoln / School of Biological Sciences. Nanotechnology is expanding rapidly, thus increasing the release of engineered nano-particles (NPs) to the aquatic environment. Sediment is likely to be the environmental compartment that is most exposed to metal-bearing NPs, and organisms that feed on sediment are likely to be particularly at risk from metal-NP exposure. Due to their increased surface area and reactivity, NPs may be more bioavailable and toxic than their macroscale counterparts. However, very little is known about the environmental impact of NPs and whether they behave similarly in the environment compared to their macroscale counterparts, and these factors make it difficult to perform environmental risk assessments and to set quality standards for NPs. Some of the questions we are facing are whether nano-specific properties lead to unexpected and new biological effects? If so, is the potential for such effects diminished when NPs are introduced to a complex environment such as the sediment compartment? Acknowledging that we are not yet able to characterize NPs once they have been introduced to the sediment compartment, we used a comparative approach to assess toxicity and bioavailability of Ag NPs. This presentation will show that by using realistic exposure scenarios, applying different well-characterized forms and sizes of Ag to the sediment compartment, and examining subsequent bioavailability and toxicity, we found evidence that bioavailability was affected by the Ag form introduced even though metal inevitably will change speciation once it enters the sediment compartment. The effect and importance of metal form added to sediment for metal bioavailability and subsequent toxicity is not straightforward, however, and depends both on biological factors, such as digestive complexity, and physical-chemical factors affecting the fate and speciation of metals in the sediment compartment. To better understand these mechanisms we need to focus future research efforts on developing methods to characterize nanomaterials in complex environments, including sediment, soil and diet, that are robust, reliable and yet relatively easy to implement in a standard laboratory.

**393 Uptake, elimination, and relative distribution of total silver in various tissues of Common Carp (*Cyprinus carpio*) after aquatic exposure of silver nanoparticles** M. Jang, J. Park, J. Oh, Y. Lee, Korea Institute of Toxicology / Future Environmental Research Center; S. Lee, Korea Institute of Toxicology / Future Environmental Research Center, Korea Institute of

Toxicology / Ecotoxicology Laboratory. Despite increasing application of silver nanoparticles (NPs) in industry and consumer products, relatively little research has been conducted on the fate, behavior and bioavailability of the materials in the water column. To investigate the fate and effects of silver NPs in fish, common carp (*Cyprinus carpio*) were exposed to water treated with silver NPs. A semi-static test system was used to expose common carp to either a freshwater control, or 0.5 mg l<sup>-1</sup> silver NPs for up to 7 days followed by 2 weeks depuration period. Uptake into the gills, brain, fillet, gastrointestinal tract, liver and blood was quantified by inductively coupled plasma-optical emission spectrometry (ICP-OES) after acid digestion (with combination of concentrated HNO<sub>3</sub> and hydrogen peroxide). Prior to experiment, recovery efficiency of silver NPs during acid digestion was tested and the percent recoveries from tissue extracts ranged from 90.9 to 111.4 %. Over the exposure period, the concentrations of total silver were stable in water as the measured concentration of silver, 0.62 ± 0.12 mg l<sup>-1</sup> (mean ± SD) was slightly higher than the nominal concentration, 0.5 mg l<sup>-1</sup>. During the uptake periods, the highest concentrations of total silver was found in the liver (5.61 mg kg<sup>-1</sup>), indicating that it is the target organ for silver NPs exposure. The concentration of total silver in gill, gastrointestinal tract, brain, fillet and blood was 3.32, 2.93, 0.14, 0.18, and 0.02 mg kg<sup>-1</sup>, respectively. After 14 days of depuration, silver were not detected in the fillet, blood and brains, however there were still detections of total silver in the liver, gastrointestinal tract, and gills. The concentrations of total silver in each tissue after 14 days of depuration were 4.22, 1.26, and 0.77 mg kg<sup>-1</sup>, respectively. These results contribute to a better understanding of the potential health effects of exposure to manufactured nanomaterials on species in aquatic ecosystems.

**394 Mechanism of Nanoparticle Silver Accumulation in *Daphnia pulex*** E. Costa, Wilfrid Laurier University; J. McGeer, Wilfrid Laurier University / Department of Biology. This research aims to understand the acute and chronic uptake and toxicity of nanoparticle forms of silver (nAg) to *Daphnia pulex* through comparisons to ionic silver (Ag<sup>+</sup>). A three-step approach considered toxicity, solution characterization, and physiological effects to determine if nAg toxicity is due to individual particles or particle dissolution to Ag<sup>+</sup>. Acute and chronic toxicity tests were standard 48 h and 21 d exposures, respectively, to determine 25% (EC25) and 50% effect concentrations (EC50). Using filtration and dissolution techniques, solutions at these concentrations were measured for size fractions (e.g. Ag content in < 450nm, < 100nm, < 10nm, and < 1nm fractions) and assessed for the relative content of nAg and Ag<sup>+</sup> associated with effects. The size fractionation and dissolution assessments were done on three solutions: water only, water + neonates (acute simulation), and water + adult *Daphnia* + food (chronic simulation). To assess physiological effects, 6 d old *Daphnia* were exposed to the chronic EC25 (0.4 µg/L Ag) to measure short-term (3 h) and long-term (48 h) bioaccumulation as well as <sup>22</sup>Na influx (to determine if nAg disrupts Na balance). Acute (EC50 0.8µg/L) and chronic (EC25 0.4µg/L) toxicity is similar for Ag<sup>+</sup> as well as three forms of nAg. However, uptake kinetics (3 h and 48 h) differed compared to Ag<sup>+</sup> as did Ag induced Na disruption (compared to Ag<sup>+</sup> and among nAg forms). Exposure solution characterization revealed that dissolution of nanoparticles to form ionic silver in solution does not play a significant role in nAg toxicity. Size fractionation of nAg particles revealed that particle size distribution is influenced by food and the presence of test organisms. This research was supported by the NRC-NSERC-BDC Nanotechnology Initiative.

**395 Silver Nanowire Toxicity on *Daphnia magna*** L.D Scanlan, U.C. Berkeley / Molecular Toxicology, U.C. Berkeley / graduate student; R. Reed, Colorado School of Mines; B. Gilbert, LBL Earth Sciences Division; N. Karunaratne, M. Eng, T. Cun, U.C. Berkeley; C. Higgins, J. Ranville, Colorado School of Mines; C. Vulpe, University of California, Berkeley / Nutritional Sciences and Toxicology. The use of nanomaterials and their subsequent release into the environment is increasing and it is therefore important to understand the toxicity of nanomaterials in ecological indicator organisms such as *Daphnia magna*. Silver nanowires (AgNW) are a high aspect ratio nanomaterial that are toxic to *Daphnia magna*. Previous experiments showed AgNW with poly(vinyl pyrrolidone) (PVP) or inorganic amorphous aluminum-doped silica (SiO<sub>2</sub>) coatings that were either 2 µm x 30 nm or 20 µm x 60 nm have toxicities in the 4 – 500 µg/L range. AgNW were detected inside *Daphnia* hemolymph following a 24-hour exposure, indicating absorption across the gut or gill epithelia. Furthermore, irregular shaped nanorods were detected in the hemolymph,

which may have formed from proteins interacting with silver species *in vivo*. To investigate environmental effects on the toxicity of AgNW, we exposed daphnids to AgNW in *Daphnia* growth media with high (8.8) and low (6.5) pH or with high (20ppm) or low (2ppm) levels of dissolved organic carbon. We also performed single particle inductively coupled plasma mass spectrometry (spICPMS) on hemolymph extracted from the *Daphnia* to verify AgNW uptake into the hemolymph and to quantitate internal silver levels. spICPMS was done on silver and AgNW-exposed animals following a post-exposure gut clearance depuration period with or without food. We found that changes in environment can affect LC<sub>50</sub>. We also found no difference in silver load between animals fed or not fed during the 1.5-hour gut clearance, which indicates the silver detected in hemolymph is not a result of puncturing the gut during hemolymph extraction. spICPMS also detected larger silver species in *Daphnia* following Ag<sup>+</sup> exposure, which indicates the animals may form biological silver nanoparticles *in vivo*.

**396 Assessing the Ecological Risk of Nano Silver: A Perspective Based on Current Knowledge** S.A Diamond, U.S. EPA / MED, U.S. EPA / Mid-Continent Ecology Division, U.S. EPA / MED. The development of nanotechnology has led to the creation of a variety of materials having unusual and potentially beneficial properties. Among these materials, nano-silver (n-Ag; having approximate dimensions between 1 and 100 nm) shows particular promise due to its ease of incorporation into fabrics, polymers, porous silica and other materials, where its antimicrobial activity can be of great economic, environmental, and societal benefit. In addition, n-Ag has relatively large surface area per volume, the potential for tightly controlled release of free silver, and exhibits novel quantum properties including strong absorbance and fluorescence signatures that indicate the potential for its use in a broad range of applications other than antimicrobial. These properties also suggest a capacity for production of ROS in some systems. Recent ecotoxicological research indicates that, in many systems (particularly aquatic) degradation of n-Ag (release of free Ag species) is the primary pathway for exposure and effects, suggesting that fate processes elucidated for ionic silver can accurately predict hazard where rates of degradation are defined. Generation of ROS has also been shown to contribute to toxicity. Fate studies have defined degradation rates and also demonstrated the concomitant generation of peroxide; which might explain ROS-related toxicity for n-Ag. All of these processes have been shown to be strongly dependant on the form or structure of n-Ag, e.g. coated, embedded, functionalized, or varying in crystal structure. These and other key risk assessment issues and regulatory challenges associated with n-Ag will be discussed relative to recent research findings.

**397 Characterization of freshwater natural dissolved organic matter (DOM): quality perspectives for direct and indirect interactions with organisms** H. Al-Reasi, McMaster University / Department of Biology; S. Smith, Wilfrid Laurier University / Department of Chemistry, Wilfrid Laurier University / Chemistry; C.M. Wood, McMaster University / Department of Biology. Direct and indirect influences on aquatic organisms in the presence of different sources of dissolved organic matter (DOM) have been revealed, but many have exhibited unexplained, source-dependent variability. As an attempt to understand the role of DOM quality, various freshwater DOMs were characterized by absorbance and fluorescence spectroscopy and potentiometric titration. Specific absorptivities at 254, 340 and 436 nm (SUVA<sub>254</sub>, SAC<sub>340</sub>, and SCOA<sub>436</sub>) were indices of aromatic carbon composition and colored content. Absorbance ratios of Abs<sub>254/365</sub> and Abs<sub>octanol 254</sub>/Abs<sub>water 254</sub> were used as molecular weight index and as octanol solubility index of DOMs, respectively. Fluorescence index was employed to classify DOMs and the excitation-emission fluorescence spectra were resolved by parallel factor analysis (PARAFAC) resulting in four fluorescent components (humic-like, fulvic-like, tryptophan-like, and tyrosine-like). Magnitude of all measures varied widely between DOM samples, indicating substantial differences in term of compositional and molecular nature and corroborating the use of absorbance and fluorescence measurements in addressing the issue of quality of heterogeneous DOMs. Aromaticity (SAC<sub>340</sub> and SUVA<sub>254</sub>), content of colored moieties (SCOA<sub>436</sub>) and the relative concentration of humic-like fluorophore showed an ascending source dependent continuum from autochthonous to terrigenous DOMs, molecular weight index (Abs<sub>254/365</sub>) and the relative concentration of fulvic-like fluorophore demonstrated a descending trend. Autochthonous DOMs had relatively higher magnitudes of octanol solubility, tryptophan- and tyrosine-like composition than the terrigenous isolates. Acid-base

titrations revealed the dominance of carboxylic and phenolic ligands with a trend for more autochthonous source to have higher total proton binding capacity. With the introduction of a summary parameter, referred to as the tridentate index, it is possible to directly relate optical characteristics, i.e., SAC<sub>340</sub>, and chemical (proton) reactivity. Metal reactivity is related to proton reactivity; thus, the tendency observed in the literature that darker organic matter is more protective in metal toxicity studies can now be rationalized on a mechanistic basis. The optical indices described above may provide clues for justifying the unsolved variations of direct and indirect influences on aquatic organisms in the presence of DOMs.

**398 Does ecosystem disturbance influence the toxicity mitigation quality of natural organic matter?** K.C Livingstone, J. McGeer, Wilfrid Laurier University / Department of Biology. Natural organic matter (NOM) has an important role in the recovery of damaged aquatic systems, particularly those impacted by metals. This aim of this study is to examine the influence of NOM source quality on acute Cu toxicity to the freshwater amphipod *Hyalella azteca*. Acute toxicity tests (96h) were done according to standard (Environment Canada) methods, with tests being completed in duplicate using 10 *Hyalella* (2-9d) added to solutions of Cu (0-4µM) and NOM sources at a DOC concentration of 5 mg C/L. NOM was collected by reverse osmosis from sites that had been disturbed by logging, fire, or long term smelter emissions. Culture and test solutions were kept at pH 7.1±0.1, 20-21°C, and hardness of 13 mg CaCO<sub>3</sub>/L. The LC50s for Cu-NOM solutions were significantly different, indicating variation in quality among NOM sources. Disturbed sites offered less protection against Cu toxicity than reference sites. Short-term (6h) Cu bioaccumulation studies were conducted to compare the effect of DOC (5 mg/L) on uptake; which varied in the severity of disturbance (undisturbed, fire, or logging). High levels of Cu were accumulated when no NOM was present and less was accumulated with NOM. The undisturbed site NOM was most effective in reducing short term Cu bioaccumulation while NOM from fire damaged and logging damaged sites were less effective, implying that both the type and severity of ecosystem disturbance has an influence on the protective capacity of NOM. Tests to compare the quality (based on toxicity mitigation) of NOM sources demonstrated clear differences that could be related to optical characteristics measured by absorbance and excitation emission matrix spectroscopy (EEMS). The absorbance of NOM sources at 340nm provided a clear correlation with the LC50 and may act as a rapid method for predicting NOM quality. EEMS, in conjunction with PARAFAC analysis, has been found to be useful in terms of modeling by taking into account the relative composition of each NOM source. Characterization of Cu-NOM solutions by ion selective electrode was used to establish links between the binding capacity and affinity of each NOM source in order to understand the role of ecosystem disturbance in protecting against Cu toxicity. An improved understanding of NOM quality can be useful in improving predictions of Cu toxicity in freshwater systems. This project is part of the TALER Research Program supported by NSERC, Vale and Xstrata.

**399 Investigations into the mechanisms of chronic nickel toxicity to freshwater pulmonate snail, *Lymnaea stagnalis*** S. Niyogi, University of Saskatchewan / Department of Biology; K.V. Brix, M. Grosell, RSMAS, University of Miami. The freshwater pulmonate snail, *Lymnaea stagnalis*, is known to be extremely sensitive to multiple metals including Ni. An EC10 of < 2 µg l<sup>-1</sup> Ni for juvenile snail growth has recently been estimated for this species, which is well below the current USEPA chronic water quality criterion for Ni. We characterized ionoregulation and acid-base balance in juvenile (~10 day post-hatch) *L. stagnalis* to understand the potential mechanisms of chronic Ni toxicity. Individual snails were exposed to an increasing range of waterborne Ni concentration (0, 1, 2, 4, 8 and 16 µg l<sup>-1</sup>, n=10 per treatment) in 150 ml polythene containers for 24 days. The exposure water was exchanged fully every day, and the snails were supplied daily with a piece of fresh lettuce as food. The results showed a dose-dependent decrease in average growth rate (g wet weight per day), with approximately 3-fold reduction at the highest exposure dose during day14-21. The net Ca<sup>2+</sup> flux rate also remained suppressed at concentrations >2 µg l<sup>-1</sup> Ni during the entire exposure period, and this effect was most pronounced on day 14 with a 55% reduction at 16 µg l<sup>-1</sup> Ni exposure. Snails were sacrificed at the end of the exposure, and hemolymph and soft tissue were collected for the analysis of essential ions, acid-base parameters (pH and total CO<sub>2</sub>), and Ni accumulation. A dose-dependent decrease in hemolymph Ca<sup>2+</sup> concentration was observed at the end of the exposure, however hemolymph Mg<sup>2+</sup> and Na<sup>+</sup>



levels remained unaltered. In contrast, both  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  concentrations in the soft tissue decreased by 25-35% at  $>4 \mu\text{g l}^{-1}$  Ni exposure. In addition, a marked dose-dependent increase in hemolymph pH was recorded, indicating alkalosis of extracellular fluid in juvenile snails following Ni exposure. In agreement with this observation, a concurrent increase in hemolymph total  $\text{CO}_2$  level was also observed at  $2\text{-}16 \mu\text{g l}^{-1}$  Ni exposure. Finally, Ni accumulation in soft tissue increased in a dose-dependent manner, and exhibited a strong negative correlation with the hemolymph  $\text{Ca}^{2+}$  level. Interestingly though, analogous experiments suggested no direct interaction of water-borne  $\text{Ni}^{2+}$  and  $\text{Ca}^{2+}$  uptake in *L. stagnalis*. Although the basis of Ni/Ca interaction remains to be elucidated, our findings suggest that Ni causes chronic toxicity to *L. stagnalis* by inducing ionoregulatory and acid-base disturbance.

#### 400 The toxicity of aluminum to 6 different aquatic species, at a pH of 6

**B.A. Stubblefield**, Oregon State University, Oregon State University / Environmental and Molecular Toxicology; **A.S. Cardwell**, Oregon State University / Faculty Research Assistant; **B.J. Adams**, Rio Tinto / Product Stewardship; **B.W. Gensemer**, GEI Consultants / Ecological Division; **E. Nordheim**, EAA; **R.C. Santore**, HDR|HydroQual, Inc. An extensive number of studies were undertaken to assist with the assessment of the impact of water chemistry on the toxicity of soluble aluminum salts. The studies consisted of a series of chronic toxicity tests conducted with freshwater organisms. Aluminum toxicity is a function of its speciation and this is a function of water pH. Previous chronic toxicity tests with aluminum were typically conducted under acidic test conditions and few studies have been conducted at pHs more typical of natural surface waters. These studies investigated the chronic toxicity of aluminum at pH 6.0 to six freshwater species. The species tested were the great pond snail (*Lymnaea stagnalis*), a rotifer (*Brachionus calyciflorus*), an aquatic oligochaete (*Aeolosoma sp.*), an aquatic plant (*Lemna minor*), and two fish, the fathead minnow (*Pimephales promelas*) and zebrafish (*Danio rerio*). Chronic test durations ranged from 48 hours to 35 days. The most sensitive species was the zebrafish ( $\text{EC}_{10}$  150.1  $\mu\text{g/L}$  total aluminum; in water of a hardness of 100  $\text{mg/L}$  as  $\text{CaCO}_3$  and dissolved organic carbon of 0.51  $\text{mg/L}$ ) based on growth effects. The least sensitive species was *Lemna minor*, with an  $\text{EC}_{10}$  of 2175  $\mu\text{g/L}$  total aluminum (in water of a hardness of 32  $\text{mg/L}$  as  $\text{CaCO}_3$  and dissolved organic carbon of  $< 0.50 \text{ mg/L}$ ) as total dry weight. The results of these tests will contribute to a greater understanding of aluminum toxicity at circumneutral pH ranges in surface waters.

#### 401 Understanding the importance of latent toxicological effects: embryonic cadmium exposure produces less fit adults in a freshwater snail

**D.A. Kimberly**, Texas Tech University / Environmental Toxicology, Texas Tech University / TIEHH; **C.J. Salice**, Texas Tech University / Environmental Toxicology, Texas Tech University / TIEHH, Texas Tech University / Department of Environmental Toxicology. Latent effects are defined here as adverse effects in juvenile or adult stages as a result of early development exposure. These effects have been documented in a multitude of taxa after exposure to toxicants including metals and pesticides. However, the extent to which latent effects alter natural populations are largely unexplored. One unexplored avenue of research is how exposure to toxicants during early development influences subsequent responses to stress in later life stages. The objective here was to understand how early life stage exposure to cadmium influences later life stage exposures to either cadmium or temperature stress in the freshwater gastropod, *Physa pomilia*. Thirty  $< 24$  hour old egg masses from laboratory reared *P. pomilia* were divided among three initial treatments including control, 2.5ppb, and 10ppb. At hatching (6-9 days), all snails were transferred to clean water. At 21 days after hatching, snails were divided among four secondary treatments, including control, 50ppb, 150ppb, and 35C. Each secondary treatment consisted of 4 replicates with four snails in each replicate. Time to first reproduction, egg mass density, hatching success and size were recorded. Overall, snails exposed to cadmium initially and temperature secondarily were most adversely affected. For example, time to first reproduction was significantly longer for animals exposed to cadmium and then temperature with the first egg mass observed around 42 days (control snails produced eggs in 30 days) from the start of the experiment. Snails exposed to cadmium initially and secondarily did not exhibit as severe adverse responses as those exposed to temperature but more severe than animals among the cadmium initial/control secondary treatments. Surprisingly, animals exposed to cadmium in the egg mass but nothing secondarily showed significant adverse effects compared to overall

controls indicating an important latent effect of early exposure to cadmium. The effects of embryonic cadmium exposure manifested across all fitness related responses. Our research shows that animals exposed in an early life stage (egg mass) can show significant latent adverse effects. Because most experiments dealing with toxicant exposures make observations over a single life stage, latent effects will go largely unnoticed. As a result, latent effects may have a large impact on future susceptibility to novel stressors and thus influence individual survival and population persistence.

#### 402 Correlating Zn and Cd elimination rate constants across taxonomic groupings

**M.D. Poteat**, North Carolina State University / Department of Environmental and Molecular Toxicology, North Carolina State University / Dept. of Environmental and Molecular Toxicology; **D.B. Buchwalter**, North Carolina State University / Department of Environmental and Molecular Toxicology, North Carolina State University / Department of Environmental & Molecular Toxicology. Biodynamic modeling has emerged as a powerful tool for understanding and predicting metal accumulation in aquatic organisms. Of the parameters that feed biodynamic models, the rate constant of loss ( $k_e$ ) has proven to be the most sensitive factor in determining metal accumulation in organisms. We measured Zn and Cd  $k_e$ 's following dissolved exposures with 65-Zn and 109-Cd in 11 species of aquatic insects representing the families Hydropsychidae and Ephemerellidae. The  $k_e$ 's ranged 9.8-fold for Zn and 14-fold for Cd and were strongly correlated across species ( $r=0.93$ ,  $p<0.0001$ ). The strength of this correlation allowed us to test predictions of Zn elimination in three additional insect species based on pre-existing data for Cd. All measured Zn  $k_e$ 's fell within the 95% confidence interval of the regression with observed values averaging 8% deviation from predicted values. To explore whether this relationship between Cd and Zn  $k_e$ 's was limited to insects, we further explored the correlation of Zn and Cd  $k_e$ 's in reported literature for 17 other species in varying taxonomic groups. A global analysis of all species shows that Zn and Cd  $k_e$ 's are well correlated whether analyzing data with conventional or phylogenetic statistics. Interestingly, different faunal groups had distinct slopes when Zn  $k_e$  was regressed against Cd  $k_e$  (aquatic insects:  $m=0.79\pm0.1$ , bivalves:  $m=1.11\pm0.2$ , and fish:  $m=0.46\pm0.2$ ), yet correlations remained high between the metals within faunal groups. Significant correlations between Cd and Zn  $k_e$ 's were found within bivalves ( $r=0.86$ ,  $p<0.0001$ ) and fish ( $r=0.65$ ,  $p=0.02$ ) when including all possible routes of metal exposure (dissolved, pulse, and dietary exposure). Exposure route appears to be an important consideration however. When only analyzing  $k_e$ 's for all organisms exposed to dissolved Zn and Cd,  $k_e$ 's were strongly correlated ( $r=0.84$ ,  $p<0.0001$ ). However, there was no correlation of Zn and Cd  $k_e$ 's for organisms exposed via diet, nor were trends apparent with respect to the magnitude of dissolved and dietary  $k_e$ 's. Certain species showed unique trends in elimination. In *Daphnia magna*, Zn  $k_e$ 's were an average of 3-fold higher than Cd  $k_e$ 's following dietary exposures and an average of 9.5-fold higher following dissolved exposures. It may be possible to predict the  $k_e$  of one metal from existing data for the other (for Cd and Zn) when taxonomic position and mode of uptake are taken into consideration.

#### 403 Intracellular Speciation of Pb in the Green Alga C. reinhardtii using High Energy Resolution Fluorescence Detected X-ray Absorption Spectroscopy

**T. Stewart**, Swiss Federal Institute of Aquatic Science & Technology / Environmental Ecotoxicology, Swiss Federal Institute of / Aquatic Science & Technology; **J. Szelachetko**, PSI, Paul Scherrer Institute / Bioenergy and Catalysis Laboratory; **R. Behra**, Eawag, Swiss Federal Institute of Aquatic Science and Technology / Environmental Ecotoxicology; **L. Sigg**, Eawag, Swiss Federal Institute of Aquatic Science and Technology / Environmental Toxicology; **M. Nachttegaal**, PSI, Paul Scherrer Institute / Bioenergy and Catalysis Laboratory. Metal bioavailability and toxicity can often be predicted by solution chemistry and related to intracellular accumulation. Use of spectroscopic methods like X-ray Absorption Spectroscopy (XAS) can help to determine intracellular metal speciation, furthering insights into mechanisms of toxicity and detoxification. High-energy resolution fluorescence detected (HERFD)-XAS was used to obtain information on the intracellular speciation of Pb in the model green alga, *C. reinhardtii*. Obtaining structural information of Pb complexes with traditional XAS spectroscopy is difficult, as the lone pair of electrons leads to poorly defined Pb-ligand bond distances. With HERFD-XAS the detection of the fine structure in the absorption edge region of the spectrum is possible. A short-term exposure (0.1  $\text{mM Pb}^{2+}$ , 10  $\text{mM MOPS}$ , pH 7) using exponentially growing cells was conducted, and an EDTA wash was used to

remove cell surface bound Pb. PbO, PbS, PbAcetate, Pb(OH)<sub>2</sub>, Pb<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub>, Pb(NO<sub>3</sub>)<sub>2</sub>, PbGly, PbCys, and PbGSH were used as references for Pb bound to different functional groups. For each compound, the Pb L<sub>3</sub> absorption edge (13000 – 13100 eV) was probed and fluorescence emission, spanning the L<sub>α1</sub> and L<sub>α2</sub> emission energies, was detected yielding full 2-D resonant inelastic X-ray scattering (RIXS) maps. The RIXS maps allow discrimination between O and S ligands. Furthermore, RIXS planes of references show unique features, allowing conclusions to be made about the type of Pb complex. Fitting of reference RIXS plane to the algae sample revealed that intracellular Pb speciation is a mix of S ligands (63%) and Pb<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub> (37%). This *in vivo* study suggests the importance of not only S ligands, but also of Pb phosphate in metal detoxification.

**404 Is Pb internalization by *Chlamydomonas reinhardtii* limited by diffusion? Comparison of observations vs. theoretical predictions** P. Sanchez Marin, Institut National de la Recherche Scientifique / Centre Eau Terre Environnement, INRS-ETE / INRS-ETE; C. Fortin, INRS – Eau, Terre et Environnement / Université du Québec, Université de Québec / INRS-ETE; P.G. Campbell, Université de Québec / INRS-ETE. The flux of lead (Pb) internalization by the unicellular alga *Chlamydomonas reinhardtii* has been observed to be surprisingly high for a non essential metal. In the presence of carbonate as an inorganic ligand and for a given [Pb<sup>2+</sup>], Pb internalization was higher than in the absence of binding ligands. Over a common range of free Pb<sup>2+</sup> concentrations, according to the biotic ligand model (BLM), similar uptake was expected. The enhanced internalization in the presence of carbonate was observed only at Pb<sup>2+</sup> concentrations lower than 50 nM. One plausible explanation for these results would be that the uptake of Pb by this alga is limited by the diffusion of Pb<sup>2+</sup> ions towards the cell surface. If the alga takes up Pb<sup>2+</sup> at a higher rate than the rate of supply of free Pb<sup>2+</sup> by diffusion, there will be depletion of Pb<sup>2+</sup> ions in the vicinity of the cell. The presence of complexes that can dissociate in the diffusion layer surrounding the alga would attenuate this depletion, therefore possibly enhancing Pb uptake. In order to check if this process is taking place for *C. reinhardtii*, three different approaches were used: (1) First of all, simple calculations of the maximum diffusive flux of Pb towards the cell surface were made to see if the predicted flux was higher or similar to the observed internalization fluxes; (2) Pb uptake in the presence of other ligands (citrate, iminodiacetate) was evaluated to see if their presence also increased Pb internalization at low Pb<sup>2+</sup> concentrations as observed with carbonate; and (3) A flow-through experiment was performed to evaluate if an increase in flow regimes – and concomitant decrease in diffusion layer thickness – had an effect on Pb internalization fluxes in the absence of ligands. The different Pb complexes tested affected Pb internalization in different ways; some of the ligands increased Pb uptake whereas others did not. The observed results are compared with theoretical predictions of Pb fluxes, considering the degree of lability of the different Pb complexes. This study will contribute to the refinement of predictive models such as the BLM.

**405 Is it time for Environment Canada to turn the page on the *Hyaella azteca* standard method?** L. Taylor, Environment Canada; J. Miller, Miller Environmental Sciences Inc.; L. Van der Vliet, P. Jackman, R. Scroggins, Environment Canada. The Environment Canada (EC) standardized test method for *Hyaella azteca* (EPS 1/RM/33; Biological Test Method: Test for Survival and Growth in Sediment using the Freshwater Amphipod *Hyaella azteca*) was originally published in 1997. Over the past fifteen years, several technical advancements have been made to the 14-day survival and growth test which requires updating the 1<sup>st</sup> edition. Most notably, the EC Atlantic Laboratory for Environmental Testing conducted experiments to refine specific aspects of the new test design for a water-only exposure (note: this type of exposure is completely new to the 2<sup>nd</sup> edition). In addition, new guidance regarding updated statistics, conducting field replicates, barcoding for taxonomic identification, the use of solvent and negative controls and the implementation of water quality thresholds for triggering renewal will be included in the new 2<sup>nd</sup> edition scheduled for release later this year. Aside from these and other technical additions, the method also requires a general overhaul. This includes for example, contrasts to other *Hyaella azteca* methods from different agencies (i.e., US EPA, ASTM, ISO) for harmonization purposes and the mutual recognition of data.

**406 Results of 10, 28, and 42-day feeding trials with *Hyaella azteca* after additions of bromide and chloride to well water** H. Krueger, Wildlife International, LTD / Aquatic Toxicology, Wildlife International, LTD;

S. Thomas, N. Billa, Wildlife International, LTD. Many laboratories are working on feeding trials with *Hyaella azteca* in order to improve survival and growth in the 10-day test and survival, growth, and reproduction in the 42-day test. The *Hyaella azteca* advisory group (HAAG) has been sharing information among labs that are working on improving these endpoints. Our lab has been working on feeding trials in formulated sediment test systems and also in water only exposures. Well water is used in these trials with additions of chloride and bromide. Before these additions were made we did not see good survival, growth, or reproduction in trials. Feeding rates also have been ramped up during trials as suggested by other HAAG trials. We have tested several types of food including YCT and Tetramin flake food in suspension. These diets have been tested with and without supplements of Navicula (a diatom). The additions of bromide to 20 ppb and chloride to 20 ppm in well water, as well as ramping up feeding rates seem to have had the most dramatic effects on survival, growth, and reproduction, since all have been greatly improved regardless of food types. Increases in growth at 10 and 28 days have easily met the new proposed criteria a 2.5x increase in weight at 10 days and a 10x increase in weight at 28 days. Results of past trials and trials currently running will be presented to evaluate the reproducibility of results.

**407 Benefits of biofilm in optimizing survival, growth, and reproduction of the amphipod *Hyaella azteca* in static-renewal, water only exposures** D. Soucek, Illinois Natural History Survey / Illinois Natural History Survey, Illinois Natural History Survey / Illinois Natural History Survey, Institute of Natural Resource Sustainability / Illinois Natural History Survey; A. Dickinson, K. Major, Illinois Natural History Survey. While the amphipod *Hyaella azteca* has been used for decades in toxicity tests, and standardized sediment toxicity testing guidelines have been developed for this species by U.S. EPA and ASTM, no standardized chronic water-only toxicity testing methods have been established. The recommended diet for sediment toxicity testing is a commercially available Yeast-Cereal leaves-Trout chow (YCT) mixture, but a wide variety of foods and test waters are used by toxicology researchers for culturing and testing of this species. This variation may be contributing to a range of culture health conditions that may lead to inconsistencies among laboratory testing results. We conducted nine 42-d static-renewal exposures with *H. azteca* using a variety of different foods as treatments to determine if there is a combination that consistently produces high survival, growth and reproduction. Our first important finding was that Nitex screens covered with a thin biofilm consisting of water mold, green algae, and stalked ciliates consistently produced high survival (>90%), growth (frequently > 0.9 mg dry weight) and reproduction (frequently > 10 young per female) with relatively low coefficients of variation, whereas using clean Nitex screens with no biofilm but otherwise the same diet produced highly variable results. While biofilm appears to be beneficial, standardization would be difficult. Therefore we tested a diet of three commercially available diatoms, added with Tetramin, as an alternative to natural biofilms, and observed survival, growth, and reproduction comparable to and in some cases greater than that observed with the biofilm treatments. The Tetramin plus diatom combination is a good candidate for a standardized diet for *H. azteca* in chronic, static-renewal, water-only toxicity tests.

**408 Evaluation and improvement of sediment and water-only exposure methods for *Hyaella azteca*** D.R. Mount, US Environmental Protection Agency / ORD; T.L. Highland, US EPA / ORD, NHEERL, Mid-Continent Ecology Division; J.R. Hockett, U.S. EPA / Mid-Continent Ecology Division; D. Hoff, US Environmental Protection Agency / ORD, NHEERL, Mid-Continent Ecology Division, Ecotoxicology Analysis Research / Mid-Continent Ecology Division; C. Jenson, U.S. EPA; T.J. Norberg-King, U.S. EPA / ORD, NHEERL, Mid-Continent Ecology Division, US EPA / Mid-Continent Ecology Division; T.W. Valenti, Syngenta Crop Protection LLC / National Research Council, National Research Council / U.S. Environmental Protection Agency. The amphipod *Hyaella azteca* is widely used as a test organism in a variety of short-term and chronic toxicity tests in both sediment and water-only exposures. Many such tests are conducted according to, or using modifications of, sediment test guidelines developed by ASTM and U.S. EPA in 2000. Recently, we have completed a number of experiments evaluating performance under those previous guidelines, and exploring ways to improve and refine test guidelines. Perhaps the most significant finding is that the feeding regime used in the existing guidelines (1 ml of yeast/cereal leaves/trout chow slurry per day per chamber) becomes limiting to *Hyaella* in exposures longer than 10 days, and much

higher growth and reproduction rates can be achieved with larger rations or alternate diets. Multiple experiments were conducted evaluating alternate foods, and various feeding rations. Given that *Hyalella* increase in mass approximately 40-fold during a 42-day chronic test, the traditional approach of feeding a single fixed ration for the entire test seems inappropriate. Using a diet comprised of wheatgrass powder and flaked fish food, we evaluated a number of profiles of increasing ration to determine regimes that would provide high rates of growth while avoiding excess feeding. These alternate feeding schedules are shown to produce considerably higher growth and reproduction rates than were typical under the previous ASTM or U.S. EPA guidance, with 42-d weights of *Hyalella* in excess of 0.8 mg dwt/individual and reproduction well over 10 young per female. Results of experiments evaluating other aspects of test procedures are also discussed and incorporated into recommendations for consideration in the refinement of future test methods for conducting water or sediment exposures with *Hyalella*.

**409 How Good Should Control Sediment Be?** P.J. Lasier, USGS-Patuxent Wildlife Research Ctr. / University of Georgia; M.L. Urich, USGS-Patuxent Wildlife Research Ctr. Acceptable performance of the freshwater amphipod, *Hyalella azteca*, in sediment exposures is based on growth within control treatments that often consist of quartz sand. However with the standard food allocation (1 ml Y-C-T/d), growth using this substrate can be inconsistent and marginal. A week-by-week comparison was conducted over 28 days to determine differences in growth between treatments of sand and sand amended with a small amount of conditioned alfalfa (0.24%), and between treatments of the standard feeding regime and one in which the allocation of food is doubled after two weeks. Length and weight were significantly greater ( $\alpha \leq 0.05$ ) in exposures to the amended sediment, and the increased food allocation resulted in significantly greater weight after 21 and 28 days and significantly greater length after 28 days. Growth of amphipods in sand exposures receiving the standard diet was only slightly above the acceptable minimum. With additional food, amphipod growth increased in sand exposures but remained well below levels observed in the amended-sand treatments. Mean length of amphipods exposed to the amended-sand treatments and fed the standard diet was similar to lengths observed in previous exposures of *H. azteca* to natural sandy sediments with similar organic content. Animals in amended-sand treatments receiving additional food grew the largest. Increasing the food ration will most likely increase amphipod growth in all treatments and maintain the disparity between control and natural sediments. Satisfactory growth that is comparable with exposures to natural sediments can be obtained with the standard diet using amended sand.

**410 Relationship of metals, bifenthrin, habitat, sediment parameters and water quality conditions to *Hyalella sp.* abundance in urban California streams** L.W. Hall, University of Maryland / Wye Research & Education Center; R. Anderson, University of Maryland. The objectives of this study were to determine the relationship between *Hyalella sp.* abundance in four urban California streams with the following parameters: (1) 8 bulk metals (As, Cd, Cr, Cu, Pb, Hg, Ni, and Zn) and their associated sediment Threshold Effect Levels (TELs); (2) bifenthrin sediment concentrations; (3) 10 habitat metrics and total score; (4) grain size (% sand, silt and clay); (5) Total Organic Carbon (TOC); (6) dissolved oxygen and (7) conductivity. California stream data used for this study were collected from Kirker Creek (2006 and 2007), Pleasant Grove Creek (2006, 2007 and 2008), Salinas streams (2009 and 2010) and Arcade Creek (2009, 2010). *Hyalella* abundance in the four California streams declined when metals concentrations were elevated beyond the TEL. There was also a statistically significant negative relationship between *Hyalella* abundance and % silt for these 4 California streams as *Hyalella* were not found in silt areas. No statistically significant relationships were reported between *Hyalella* abundance and metals concentrations, bifenthrin concentrations, habitat metrics, % sand, % clay, TOC, dissolved oxygen and conductivity. The results from this study highlight the complexity of assessing which factors are responsible for determining the abundance of amphipods, such as *Hyalella sp.*, in the natural environment.

**411 An evaluation of the sensitivity of lab-reared and field-collected *Hyalella azteca* to the pyrethroid insecticide bifenthrin** S. Clark, S. Ogle, A. Gantner, Pacific EcoRisk; G. Mitchell, FMC Corporation. *Hyalella azteca* are epibenthic macroinvertebrates commonly used for sediment toxicity studies. *H. azteca* has also recently seen considerable use in toxicity

tests of water samples in California, as the organisms are reported to be more sensitive to pyrethroid insecticides than other routine invertebrate toxicity test species (e.g., *Ceriodaphnia dubia*). Resident *H. azteca* are known to occur, at times in abundance, in some sediments containing pyrethroid pesticides concentrations that are toxic to lab-reared *H. azteca*. This suggests potential significant differences in the sensitivity of lab-reared and resident field populations of *H. azteca* to pyrethroids. The goal of this study was to determine the sensitivity of various *H. azteca* populations, including field populations, to the pyrethroid bifenthrin. For the first phase of this study, *H. azteca* were collected from Pleasant Grove Creek, a Northern California site with a historical record of robust populations of *H. azteca* and which is dominated by residential land use. Lab-reared *H. azteca* were obtained from a widely used commercial vendor, and were 7-14 days old (and within 2 days of age) when initiating testing. Since the field organisms could not be aged, they were sieved to obtain a size close to that of the lab-reared organisms. The *H. azteca* were exposed to nominal concentrations of 0.89, 2.7, 8, 24, and 72 ng/L bifenthrin in water for 96-hours (the reported 96-hr LC<sub>50</sub> for bifenthrin toxicity to *H. azteca* is 7.7 ng/L). The resulting LC<sub>50</sub> was 10.6 ng/L and >72 ng/L for the lab and field *H. azteca*, respectively. Based on these results, a follow-up exposure was performed with higher concentrations of bifenthrin. The resulting LC<sub>50</sub> was 2.7 ng/L and >388 ng/L for the lab and field *H. azteca*, respectively, a difference of greater than two orders of magnitude. These results demonstrate that lab-reared *H. azteca* were far more sensitive to bifenthrin than the field-collected organisms. In order to determine how common such differences in sensitivity are, testing is underway with *H. azteca* collected from additional sites influenced by urban and agricultural runoff, as well as from remote sites that are not expected to receive urban and/or agricultural runoff. In addition, the sensitivity of multiple sources of lab-reared *H. azteca* cultures is being evaluated.

**412 Variation in contaminant sensitivity among *Hyalella azteca* from different sources** D. Weston, University of California / Department of Integrative Biology; H.C. Poynton, US Environmental Protection Agency / Molecular Indicators Research, University of Massachusetts-Boston / Environmental, Earth and Ocean Sciences; G. Wellborn, University of Oklahoma; M. Lydy, Southern Illinois University. There have been reports of water or sediment samples causing toxicity when tested in the laboratory with *Hyalella azteca*, yet the same waterbodies contain thriving populations of the same species. To further explore these reports, the sensitivity of ten populations of *H. azteca* to pyrethroid insecticides was determined. Three populations were from established laboratory cultures, and seven were from wild collections. The three lab cultures, and some of the wild populations, shared comparable sensitivity to pyrethroids, with LC50s within a factor of three of one another. Yet some field populations had LC50s two orders-of-magnitude greater. The relative sensitivity to pyrethroids did not appear to be a function of the extent of prior pyrethroid exposure within the habitat from which they were collected. However, pyrethroid sensitivity was related to the degree of genetic similarity among the populations. DNA sequencing was consistent with previous findings that *H. azteca* represents not a single species, but a species complex. Based on the genetic data, four members of this complex appeared to be represented within the ten populations we examined, and one member in particular was extraordinarily tolerant of pyrethroids. These findings have ramifications for use of *H. azteca* in toxicity testing, related to the source of the animals used, and how cultures are maintained.

**413 Agrichemicals and Sediments: The Hourglass** A.S. Kolok, University of Nebraska Medical Center / Environmental, Agricultural and Occupational Health, University of Nebraska – Omaha / Nebraska Watershed Network, University of Nebraska – Omaha / Department of Biology; S. Bartelt-Hunt, University of Nebraska – Lincoln; M. Sellin, Department of Zoology. Research in two watersheds within Nebraska over the past few years suggests that the fate and transport of agrichemicals is different from that for estrogenic contaminants being released from wastewater treatment plants. Wastewater treatment plants continually release estrogenic compounds into the receiving waters. As such, the endocrine disrupting compounds (EDCs) are pseudo-persistent in the waters downstream from the discharge. These compounds may be subject to biodegradation, however their concentration in the water may not decrease over time, as the compounds are continually recharged by the additional, continuous discharge. As such, the chemical plume may resemble an "eternal flame" of discharge as it moves downstream. In contrast, agrichemical contaminants are only released from fields



during storm events. EDCs will only be found in the receiving streams for only a short time after a storm event. As such, it would seem that the agricultural exposure to the local biota would be limited to a relatively short period of time. Recent research in our laboratory has suggested that in environments with episodic pulses of water-borne agricultural chemicals, the sediment plays a very important role. During the chemical pulse, the sediment loads with agricultural chemical, which then gradually desorbs from the sediment and re-enters the water in a bio-available form. Rather than being pseudo-persistent, chemicals in this environment are only episodically available to the local biota, but this episodic exposure lasts longer than expected due to the role of the sediment as both a sink and a source. Conceptually, the sediment resembles an hourglass, where a storm event can load the sediment with chemical (the resetting of the hourglass) that gradually desorbs and moves downstream (much the same as sand moving from the upper to the lower bulb within an hourglass). Previously published data from our lab and others will be presented to substantiate this conceptual model.

#### 414 An Evaluation of Linking QSARs with Interspecies Correlation Estimation (ICE) Models to Develop Screening Level Water Quality Criteria

**S.D. Dyer**, The Procter & Gamble Company / Central Product Safety, The Procter & Gamble Company / Miami Valley Innovation Center, The Procter & Gamble Company / Miami Valley Labs; S.E. Belanger, The Procter & Gamble Company / Environmental Stewardship Organization, The Procter & Gamble Company / Central Product Safety Department; J. Chaney, M. Fan, The Procter & Gamble Company; K. Mayo, USEPA; S. Raimondo, United States Environmental Protection Agency / Gulf Ecology Division, National Health and Environmental Effects Laboratory, US EPA / Gulf Ecology Division; M.G. Barron, US EPA / Gulf Ecology Division; F. Wu, State Key Laboratory of Environmental Criteria and Risk Assessment. Interspecies Correlation Estimation (ICE) models have been developed and North American species models are publicly available via [www.epa.gov/ceampub/fchain/webice/](http://www.epa.gov/ceampub/fchain/webice/). Until this year, aquatic models were restricted to invertebrates and fish species; however, several algal ICE models are now being added. ICE models provide the unique opportunity to predict several species toxicity values based on a surrogate species toxicity value. Including the predicted species toxicity values in a species sensitivity distribution (SSD), one can predict the 5<sup>th</sup> percentile hazard concentration (HC5). For the first time, HC5s can be developed for all three trophic levels based on ICE models. Furthermore, predicted species values for all 3 trophic levels can be combined to creating an "ecosystem" HC5. In this presentation we specifically explore the use of ICE models for chemicals that are known to be especially toxic to each respective trophic level. While the use of ICE models provides an alternative to deriving predicted no effect concentrations (PNECs) without an assessment factor, the potential to link ICE to QSARs (such as Ecosar) provides another opportunity to explore the development of screening level water quality criteria (PNECs) based on minimal datasets. Here again we explore the use of QSARs that clearly show trophic sensitivity, hence its effect on trophic HC5s and ecosystem HC5s. Since Ecosar provides both acute and chronic values, we compare predicted acute and chronic SSDs and HC5s to measured SSDs and resultant HC5s.

#### 415 All ER Agonists Are Not Created Equal: Weaknesses of *in vitro* EEQs as Predictors of *in vivo* Estrogenic Effects in Fish

**E. Yost**, S.W. Kullman, North Carolina State University / Environmental and Molecular Toxicology. Activation of the estrogen receptor (ER) is a common mode of action for many endocrine-disrupting compounds, including steroid hormones as well as numerous xenobiotics. The estrogenic activity of compounds with this mode of action may be estimated using *in vitro* bioassays, such as the yeast estrogen screen (YES), which utilizes 17 $\beta$ -estradiol (E2 $\beta$ ) as a standard and reports the estrogenicity of a sample in terms of E2 $\beta$ -equivalents (EEQ) based on ligand interaction with the human ER  $\alpha$ . Assays such as the YES are useful as a high-throughput means of screening chemical compounds and environmental samples for estrogenic activity; however, there is concern that there may be a discrepancy between *in vitro* EEQs and actual toxic effects in aquatic organisms. In this study, we tested the hypothesis that YES-derived EEQs should be able to accurately predict the effects of estrogenic compounds on exposed male Japanese medaka, using the expression of estrogen-responsive genes (vitellogenin, choriogenin, and ER  $\alpha$ ) as a measure of estrogenic effects. Male medaka were exposed for 7 days to E2 $\beta$  at a concentration of 200ng/l, or to a suite of steroidal estrogens [estrone (E1), 17 $\alpha$ -estradiol (E2 $\alpha$ ), estril (E3)] or an environmental sample (effluent from an industrial swine operation) at

a YES-derived EEQ of 200ng/l. We found that these exposures did not elicit equal gene expression responses in the male medaka hepatocytes. The expression of estrogen-responsive genes was significantly lower in the E1-, E2 $\alpha$ -, and swine effluent-exposed fish, compared with fish that were exposed to E2 $\beta$  or E3. *Ex vivo* exposure of medaka livers to these same treatments elicited the same hepatic gene expression profiles as the whole-fish exposures, indicating that the discrepancy between the YES-derived EEQ and *in vivo* transcriptional effects in the medaka is likely not attributable to differences in absorption, distribution, metabolism, and excretion (ADME) of these compounds. Alternatively, these differences in gene expression may be attributable to molecular level events associated with differential receptor binding and transcriptional regulation in the Japanese medaka. These data indicate a significant discrepancy between YES-derived EEQs and the ability of to evoke estrogenic responses *in vivo*.

#### 416 Insights into the mechanisms of androgen-induced anal fin elongation in a potential bioindicator organism, the mosquitofish (*Gambusia spp*)

**E.K Brockmeier**, University of Florida / Physiological Sciences; Y. Ogino, T. Iguchi, Okazaki Institute for Integrative Bioscience; N.D. Denslow, University of Florida / Department of Physiological Sciences and Center for Environmental and Human Toxicology, University of Florida / Dept. of Physiological Sciences &. Bioindicator organisms can be used as sensitive indicators of the ecosystem level impacts of chemical exposure through the measurement of molecular or physiological endpoints. Currently, there is no bioindicator organism of androgen exposure for use in North America. The Eastern and Western mosquitofish (*Gambusia holbrooki* and *G. affinis*) have the potential to become such a bioindicator, since males have an elongation of the anal fin (gonopodium) whose formation is driven by androgens. Female mosquitofish do not have this anal fin elongation but the gonopodial structure can be induced by androgen exposure. To gain crucial knowledge for bioindicator development, we evaluated the expression patterns of three genes in the anal fins of androgen-exposed female mosquitofish: sonic hedgehog (shh), muscle segment homeobox C (msxC) and fibroblast growth factor receptor 1 (fgfr1). These genes have known roles in zebrafish fin growth and knowledge of their expression patterns during androgen exposure can provide insight into the genetic mechanisms of action of androgen-induced anal fin elongation. Adult female mosquitofish were exposed to the vehicle control or the potent androgen receptor agonist 17 $\beta$ -trenbolone (TB) at a dose that was able to induce anal fin elongation after 2 weeks of exposure (1  $\mu$ g TB/L). Spatial gene expression in the anal fin was determined qualitatively using whole mount *in situ* hybridization. Shh was strongly expressed at the distal tip of the anal fin rays of the gonopodium at 7, 14, and 21 days of TB exposure. MsxC and fgfr1 had a wider range of expression along the same anal fin rays but with stronger expression at day 10 and weaker expression at day 5. Because of its consistent expression during androgen exposure, the full coding sequence of shh was obtained to gain insight into this gene's function, its relationship to other species' shh proteins, and how it can be used in connection with anal fin elongation as a way to evaluate androgen exposure. The putative protein sequence was 76% homologous to zebrafish ShhA and over 80% similar to fugu fish and flatfish shh. Higher homology was seen in the N-terminal region where the active protein product is located (< 90% for zebrafish, fugu fish, and flatfish). This work provides insight into the mechanisms of action of androgen-induced anal fin elongation and can serve as a foundation for future work into developing a bioindicator of androgen exposure.

#### 417 Potential Role of the Gastric Sodium Iodide Symporter in Determining the Sensitivity of African Clawed Frog (*Xenopus laevis*) Tadpoles to Perchlorate

**J. Carr**, Texas Tech University / Biological Sciences Department; S. Murali, M. Wages, Texas Tech University; D.L. Carr, Texas Tech University / Biological Sciences; B. Butler, E. Smith, Texas Tech University. Perchlorate is a potent blocker of iodide transport by the sodium iodide symporter. Data from our laboratory suggest that there is a dramatic decrease in the sensitivity of African clawed frog tadpoles (*Xenopus laevis*) tadpoles to perchlorate inhibition of thyroid function and metamorphosis between Nieuwkoop-Faber stages 49-55. Presently, nothing is known about the physiological mechanisms underlying changes in the developmental sensitivity to perchlorate. We hypothesize that the availability of iodide from non-thyroid iodide transporting tissues increases between stages 49-55 and that increased iodide delivery to the thyroid mitigates the adverse effect of perchlorate. To test this hypothesis, we re-evaluated which epithelial and non-epithelial tissues express the NIS gene and protein based upon RT-PCR

and perchlorate-sensitive 24 h [ $^{125}\text{I}$ ] whole animal uptake studies in stage 58-60 *X. laevis* tadpoles. RT-PCR analysis revealed that NIS was expressed in stomach and small intestine in addition to the thyroid gland. NIS mRNA was not detected in lung, kidney, skin, gill, muscle, heart or liver. Perchlorate sensitive [ $^{125}\text{I}$ ] uptake was found in stomach, lung, kidney, and small intestine but not gill, muscle, liver, or heart. Perchlorate-sensitive [ $^{125}\text{I}$ ] uptake by stomach was 6-10 times greater than in any other non-thyroidal tissue. While NF stage 49 tadpoles exhibited perchlorate-sensitive uptake in stomach it was roughly 4-fold less than that observed in NF stage 55 tadpoles. Although expression of the NIS gene was greater in NF stage 55 compared to NF stage 49 tadpoles this difference was not statistically significant. We conclude that gastric iodide uptake increases between NF stages 49 and 55, possibly due to post-translational changes in NIS glycosylation or trafficking within gastric mucosal cells. These developmental changes in gastric NIS gene expression may affect iodide availability to the thyroid gland. (Supported in part by the U.S. Department of Defense, through SERDP under a Cooperative Agreement with the USAF, Inst. for Environ. Safety, and Occup. Health, Brooks AFB, TX).

#### **418 Physiological and reproductive effects of Se in rainbow trout, brook trout, and cutthroat trout – a comparative multigenerational study** N. Pilgrim, J. Rasmussen, University of Lethbridge; V.P. Palace, Department of Fisheries & Oceans Canada, Stantec Consultants; L. Ripley, Alberta Sustainable Resource Development; A. Hontela, University of Lethbridge / Dept. of Biological Sciences.

Selenium (Se), an essential element, toxic at concentrations only slightly higher than those required for homeostasis, can be a concern in the aquatic environment. Selenium can cause oxidative stress and malformations in salmonids, and the toxicity is species-specific. This study investigated the effects of chronic diet exposure to Se methionine (SeMet) at 15 µg/g and high 40 µg/g, on Se accumulation in eggs, liver and muscle, and physiological and reproductive endpoints in adult female rainbow trout, brook trout, and cutthroat trout. Adults were fed the experimental diets for 5 months prior to spawning, eggs were collected and fertilized, and adult tissues sampled. Larvae were raised in a vertical incubator until yolk absorption. Brook trout had a higher ratio of liver and muscle Se to egg Se than rainbow trout or cutthroat trout, and a higher ratio of plasma T3:T4 with increasing Se in liver and muscle. Species-specific differences were also detected in relationship between tissue Se and gonadal somatic index (GSI) and oxidative stress endpoints. Lipid peroxidation (LPO) and glutathione (GSH) increased with increasing liver Se in rainbow trout, GSH increased in cutthroat trout, but there was no significant relationship between liver Se and LPO or GSH in brook trout. Egg Se burdens were correlated with a decrease in survival in rainbow trout and cutthroat larvae at all developmental stages, while brook trout only had decreased survival in the first developmental stage (spawned eggs to eyed eggs). This study provides new information about species differences in sensitivity to Se and reproductive effects. (Funded by NSERC MITHE-SN and Alberta Conservation Association).

#### **419 Effects of dietary selenium exposure on swimming performance and energy homeostasis in juvenile fathead minnow** L. McPhee, University of Saskatchewan / Toxicology Centre; D. Janz, University of Saskatchewan / Toxicology Centre, University of Saskatchewan / Veterinary Biomedical Sciences.

Selenium (Se) is known to cause chronic toxicity in aquatic species. In particular, dietary exposure of fish to selenomethionine (SeMet), the primary form of Se in the diet, is of concern. Recent studies suggest that chronic dietary exposure to SeMet alters energy and endocrine homeostasis in adult fish. However, little is known about the direct effects of dietary SeMet exposure in juvenile fish. The objective of the present study was to investigate sublethal physiological effects of dietary SeMet exposure in juvenile fathead minnow (*Pimephales promelas*). Twenty days-post-hatch fathead minnow were exposed for 60 days to different measured concentrations (2.8, 5.4, 9.9, 26.5 µg Se/g dry weight) of Se in food in the form of SeMet. After exposure, samples were collected for trace metal analysis and fish were subjected to a swimming performance challenge (critical swimming speed, Ucrit). A decrease in critical swimming speed (Ucrit) occurred at the 9.9 and 26.5 µg Se/g exposure groups compared to the control group. An increase in oxygen consumption (MO<sub>2</sub>) and cost of transport (COT) was observed in the 9.9 and 26.5 µg Se/g exposure groups compared to the control group. The active metabolic rate of the high dose group was increased relative to control, however this did not translate into a significant increase in the aerobic scope. Energy storage capacity was measured via whole-body

glycogen and triglyceride concentrations. Triglyceride concentrations in non-fatigued fish were elevated in the 5.4 µg Se/g group relative to controls. Swim motion, as well as energy storage capacity will be measured in fatigued fish and the stress biomarker cortisol will also be determined. The results will then be compared between exposure groups as well as between swam and un-swam fish to gain new insights into the sublethal effects of dietary Se exposure on a juvenile fish species.

#### **420 From Cell Lines to Fish: Ozonation as a Treatment Option for the Detoxification of Oil Sands Process Affected Water** S.B. Wiseman, University of Saskatchewan / Toxicology Centre, University of Saskatchewan / Post-Doctoral Fellow; Y. He, University of Saskatchewan / Toxicology Centre, University of Saskatchewan / PHD student; J. Anderson, S. Paterson, University of Saskatchewan / Toxicology Centre; P. Jones, University of Saskatchewan / School of Environment and Sustainability, University of Saskatchewan / School of Environment and Sustainability and Toxicology Center; K. Liber, M. Hecker, University of Saskatchewan / Toxicology Centre; L. Perez-Estrada, University of Alberta / Department of Civil and Environmental Engineering; J.W. Martin, Trent University / Division of Analytical and Environmental Toxicology; J.P. Giesy, University of Saskatchewan / Biomedical Veterinary Sciences & Toxicology Centre, Michigan State University / Department of Animal Science, University of Saskatchewan / Toxicology Centre.

In accordance with a policy of zero discharge, oil sands process-affected water (OSPW), produced by the oil sands industry in North Eastern Alberta, Canada, is stored in on-site tailings ponds. OSPW is acutely and chronically toxic to aquatic organisms. Ozonation is one possible method for remediation of OSPW as it can reduce concentrations of dissolved organic compounds, including naphthenic acids (NAs). However, given that mechanism(s) of toxicity of OSPW are not known and considering the complex exposure scenario of OSPW, studies are required to 1) determine the mechanisms of toxicity of OSPW, 2) determine the effectiveness of ozonation in reducing the toxicity of OSPW, and 3) ensure that ozonation does not cause formation of byproducts that impart toxicity upon OSPW. To address these needs, we have been utilizing a battery of *in vitro* and *in vivo* assays. Untreated and ozone-treated OSPW was characterized for endocrine disrupting properties and acute and chronic toxicity by quantifying survival, growth, and development of the benthic invertebrate *Chironomus dilutus* and the fathead minnow (*Pimephales promelas*). High-throughput sequencing of RNA to investigate gene expression in *Chironomus dilutus* and fathead minnows exposed to untreated OSPW and ozone-treated OSPW were utilized to determine mechanisms of toxicity of OSPW and to characterize the effectiveness of ozonation in attenuating toxicity. Using the H295R, T47D, and MDA cell assays we demonstrated that untreated OSPW altered synthesis of the sex steroids estradiol and testosterone as well as signaling by the estrogen receptor and androgen receptor. Endocrine disrupting effects of untreated OSPW were either attenuated or unaffected by ozonation of the OSPW. Exposure to untreated OSPW or ozone-treated OSPW did not significantly affect survival of *Chironomus dilutus*. However, growth, emergence, and pupation were significantly less in animals exposed to OSPW compared to controls, and ozonation attenuated these effects. Fish exposed to untreated OSPW up regulate several pathways related to the detoxification of xenobiotics as determined by RNA sequencing analysis. Oxidative stress and apoptosis were prominent in minnows exposed to untreated OSPW and might contribute to toxicity. These effects were not evident in fish exposed to ozone-treated OSPW. Results to date suggest that organic compounds in OSPW mediate the toxic effects of OSPW and that ozonation attenuates most of the toxicity of OSPW.

#### **421 Fundamental Studies on the Decontamination and Detoxification of Oil Sands Process-Affected Water Using Advanced Oxidation Processes** A. Afzal, N. Wang, M. Islam, L. Perez-Estrada, P. Drzewicz, P. Chelme-Ayala, University of Alberta / Department of Civil and Environmental Engineering; J.W. Martin, University of Alberta / Department of Laboratory Medicine and Pathology; M. Gamal El-Din, University of Alberta / Department of Civil and Environmental Engineering.

The oil sands industry produces large volumes of oil sands process-affected water (OSPW) as a result of bitumen extraction and upgrading processes. OSPW refers to the water that has been in contact with oil sands or released (dewatered) from tailings deposits and retained in settling basins. OSPW is a very complex mixture of suspended solids, salts, inorganic compounds, dissolved organic compounds, and trace metals. Currently, OSPW is retained on site and there is no active return to the regional watershed. To

address the environmental issues associated with the recycling and/or safe release of the OSPW into the receiving environment, engineered water treatment technologies are required. Although efforts have been made to assess individual technologies to remove or degrade specific constituent(s) of OSPW, a strategy to treat and manage the various streams of OSPW has not been proposed so far. There is an urgent need for research to find different approaches and strategies for OSPW treatment and management. Advanced Oxidation Processes (AOPs) are the chemical treatment methods designed to degrade organic and inorganic compounds by generating powerful oxidants such as hydroxyl radicals. AOPs, including ozonation, ultraviolet light (UV) combined with hydrogen peroxide ( $H_2O_2$ ), and vacuum UV, among others, are being used as treatment alternatives to accelerate the detoxification of OSPW. Moreover, AOPs are being used as pre-treatment steps in order to convert initially persistent organic compounds such as naphthenic acids (NAs) into more biodegradable intermediates, which can be treated using biological processes or other technologies. Our results show that ozone preferentially reacts with NAs with higher cyclicity. In addition, NAs with more rings and more carbon atoms are degraded more rapidly during the ozonation process. By using UV/ $H_2O_2$ , it has been found that NAs with more carbon atoms are favoured for degradation. However, increasing the number of rings (or double bond equivalents) in NAs does not show any clear structure reactivity. By using a model NA compounds (i.e., cycloheptanoic acid; CHA), it has been found that the principal byproducts formed during hydroxyl radical induced decomposition of CHA are hydroxy-CHA, dihydroxy-CHA, and oxo-CHA. This presentation is designed to bring forward the recent advances of AOPs in terms of process fundamentals, oxidation mechanism, and byproduct formation when applied to the remediation of OSPW.

**422 Gas production and gas induced transport of contaminants in tailing ponds** A. Wijdeveld, W.v. Kesteren, DELTARES; J. Foght, University of Alberta / Biological Sciences. Gas production plays an important role in the consolidation behavior of fine sediments in tailing ponds. Gas induced transport in the upper meters of the pond can enhance consolidation due to increased dewatering caused by the release of produced gas. At the same time, gas production can inhibit further strengthening of the sediment at greater depths (>10 meter) due the lack of release mechanisms and therefore the increase in gas volume in the sediment. We will present results of the large scale testing of gas induced transport and the resulting consolidation in fine sediment in a column of 15 meter in height. We also established the gas production rate in the sediment under different conditions. Combining gas induced transport measurements and gas production measurements we developed and calibrated a numeric model to predict the development of the sediment gas accumulation over a period of 1.000 years. Gas induced transport also impacts the leaching of contaminants of the pond sediment. Not only by the enhanced consolidation water flux (increased flux of dissolved contaminants), but also by the release of the gas itself. We measured the flux and composition of the gas coming from the sediment to establish the role of gas production and emission on the leaching of volatile organic compounds (VOC's) to the surface water of the pond. Most of the gas production is the result of microbiological degradation of organic compounds present in the tailing. But the gas phase itself can be an energy source for bacteria. Results based on the gas composition, SEM, confocal microscopy and selective extraction and RNA sequencing of bacteria in or around a gas bubble will be presented.

**423 Using the SPEAR approach to identify oil sands related effects on aquatic invertebrate communities** N. Gerner, UFZ Leipzig; M. Kone, University of Alberta / Civil & Environmental Engineering; A. Ulrich, University of Alberta; M. Liess, Helmholtz centre for environmental research – UFZ / Department of System Ecotoxicology, UFZ Center for Environmental Research / Department of Chemical Ecotoxicology, Helmholtz centre for environmental research – UFZ / Department of System Ecotoxicology. The Athabasca River and its tributaries are running through the Cretaceous McMurray Oil Sands Formation and are directly exposed to potential contamination via natural erosion. As a result of constantly growing mining operations along the Athabasca River, an increased input of toxic oil constituents is expected, which has the possibility to affect water quality and the environment. Current monitoring practices have been criticised for not allowing a reliable assessment of oil sands related impact. This implies the urgent necessity for an effective monitoring method that can discriminate between toxicants' effects and confounding factors. Our project aims

at developing a bioindicator system for the improvement of environmental monitoring methods in order to enable the detection of possible effects of oil sands on the aquatic ecosystem. For this purpose, the SPEAR index (SPeCiesAtRisk), a bioindicator based on the sensitivity of freshwater macroinvertebrate communities towards toxicants of interest, will be adapted to Canadian conditions. For this it will be specified for the substance groups of concern. Two sampling campaigns have been conducted in September of 2010 and 2011 and one will be conducted in September of 2012. Nine and nineteen sites have been investigated in 2010 and 2011 including references sites and sites located upstream and downstream of oil sands developments. Their macroinvertebrate communities were sampled, and in addition, water and sediment samples were taken and analyzed for major ions, polycyclic aromatic hydrocarbons (PAHs), and metals. Results of the 2010 sampling campaign indicated a negative relationship between PAH load and the community sensitivity. Thus, a decrease in the part of sensitive species in macroinvertebrate communities coincides with an increase in PAH load. The samples collected in 2011 had low PAH levels, which might be due to differences in hydrological conditions between the years. Data analysis will be expanded and the relationships between community sensitivity and toxicant load will be compared and discussed with regard to PAH and to metal contamination in 2010 and 2011. This study is expected to help achieve a more holistic understanding of potential effects of oil sand development on the aquatic ecosystem and can be implemented into current monitoring programs undertaken by environmental agencies and consultant companies.

**424 Trends in Atmospheric Deposition of Inorganic Contaminants to the Alberta Oil Sands Region Obtained from Snowpack and Lake Sediment Core Measurements** J.L. Kirk, Environment Canada / Research Scientist, Environment Canada / Aquatic Contaminants Research Division, Environment Canada; D. Muir, Environment Canada / Aquatic Ecosystem Protection Research Division, Environment Canada / Aquatic Contaminants Research Division; A. Gleason, X. Wang, F. Yang, J. Coty, A. Thawer, G. Lawson, A. Sett, Environment Canada. Atmospheric deposition is likely an important source of contaminants to the Alberta Oil Sands region. Kelly et al. (*PNAS vol 107, 2010*) demonstrated that the Oil Sands industry releases 13 elements (Sb, As, Be, Cd, Cr, Cu, Pb, Hg, Ni, Se, Ag, Tl, and Zn) considered priority pollutants (PPEs) under the US Environmental Protection Agency's Clean Water Act, via air and water, to the Athabasca River and its watershed. In spring 2011-2012, we collected snow and lake sediment cores from the Alberta Oil Sands Region to determine current and long-term trends in atmospheric deposition of 44 elements. In 2011, similar to Kelly et al., we collected snow from 30 sites located 0-200 km from the major bitumen upgrading facilities at maximum snowpack depth to quantify atmospheric contaminant loadings to the Athabasca River and tributaries. In spring 2012, this survey was expanded to ~90 sites and included the ~30 2011 sites, ~50 sites situated along transect lines within a 60 km radius the upgraders, and 8 sites in the Peace Athabasca Delta. Our 2011 results demonstrate that aerial loadings of all 13 PPEs were 1.5-13X higher at sites within 50 km of the upgraders than those >50 km away and were highest within 10 km of the upgraders. Loadings of As, Cr, Ni, and Zn were especially high, reaching up to 360, 1170, 1120, and 3270  $\mu g m^{-2}$ , respectively. Loadings of Co, Mn, and V, which were reported to the National Pollutant Release Inventory as being released in high quantities, were also elevated within 50 km of the upgraders, reaching up to 480, 22160, and 7150  $\mu g m^{-2}$ , respectively. Loadings of particulate-bound methyl Hg (MeHg) increased exponentially with proximity to the upgraders, which is important because MeHg is a neurotoxin that bioaccumulates through food-webs. Numerous crustal and rare earth elements, such Al, Ca, Fe, Ce, La, and Y showed a similar deposition pattern suggesting that in addition to the upgraders, land disturbances such as mining and land clearing are important sources of airborne contaminants. Our results support Kelly et al.'s findings that the bitumen upgraders and local Oil Sands development are sources of airborne emissions to the Alberta Oil Sands Region. 2012 snow survey results as well as results from analysis of highly-resolved, well dated lake sediment cores spanning the last 50-100 years from lakes within a 35-km radius of the upgraders will be presented to place modern contaminant deposition in the context of long-term trends.

**425 Larval Fish Toxicity of Snow Melt Waters from Oil Sands Areas** J.L. Parrott, Environment Canada / Environment Canada, National Water Research Institute / Environment Canada; W.P. Norwood, Environment Canada / Aquatic Ecosystems Protection Research Division; P.L. Gillis, J.V.



Headley, M. Hewitt, J. Kirk, R.A. Frank, J.R. Marentette, M.E. McMaster, Environment Canada; D. Muir, Environment Canada / Aquatic Ecosystem Protection Research Division, Environment Canada / Aquatic Contaminants Research Division; Z. Wang, Environment Canada. Embryo-larval fathead minnows were used to assess the toxicity of snow melt samples collected in the vicinity of the Canadian oil sands process facilities along the Athabasca River in the area of oil sands development and upstream. Snow samples were collected early March 2011 and 2012, and then shipped frozen back to the laboratory. Because the snow melt waters were low in essential ions, they were amended with several salts to mimic major ion levels up to those observed in the Athabasca River. Fertilized fathead minnow eggs were exposed (through hatch to 7-15 days post-hatch) to 25 %, 50 % and 100 % amended snow melt waters. Snow samples from upstream or far downstream of the oil sands were not toxic to larval fathead minnows at 100%. Three snow samples from around the oil sands mining and refining areas were toxic to larval minnows at 25-100 %. There was very little toxicity in larval mussels or *Hyalella* exposed to snow melt. Although pure melted snow caused effects in larval fish, samples of Athabasca River water collected just after snowmelt in 2010 caused no effects. Snow was analyzed for metals, naphthenic acids, and PAHs. Compared to other sites, the most toxic snow melt samples had higher concentrations of C1-C4 polycyclic aromatic hydrocarbons and metals, most likely from airborne deposition.

**426 Monitoring the aquatic environment in the Alberta Athabasca Oil Sands using reproductive responses in small forage fish species** M.E. McMaster, Environment Canada / National Water Research Institute; G.R. Tetreault, University of Waterloo / Biology; J. Bennett, M. Hewitt, Environment Canada / National Water Research Institute; T.W. Clark, Environment Canada; R.A. Frank, Environment Canada / AEPRD, Environment Canada / Dept. of Env. Biology & Toxicology, University of Guelph / Dept. of Env. Biology & Toxicology; J.L. Parrott, Environment Canada, National Water Research Institute. Fish were initially collected from the Steepbank River which runs through the Athabasca Oil Sands deposit, including sites off the oil sands deposit as well as within during the fall of 1999 and 2000. These fish were either unexposed, exposed to naturally occurring oil sands related compounds, or exposed to a combination of natural and anthropogenic inputs. The main objective of the study was to determine whether links with the oil sands deposit influenced baseline reproductive parameters associated with physiological or biochemical disruption in wild fish. Field collections for reproductive assessment included sampling of local fish for gonadal somatic index (GSI) as well as *in vitro* steroid production potential. These results were supported by hepatic (ethoxyresorufin-o-deethylase, EROD) activity as an indicator of exposure to oil sands related compounds. Follow-up studies were conducted during the fall of 2010-11 at the same locations to evaluate the earlier responses following additional industrial development on this tributary of the Athabasca River. Water and sediment chemistry and toxicity were also taken at these locations.

**427 A legacy of a half-century of oils sands development: the PAH and paleolimnological record in lake ecosystems** D. Muir, Environment Canada / Aquatic Ecosystem Protection Research Division, Environment Canada / Aquatic Contaminants Research Division; J. Kurek, Queen's University / Department of Biology; J.L. Kirk, Environment Canada / Research Scientist, Environment Canada / Aquatic Contaminants Research Division, Environment Canada; M. Evans, X. Wang, Environment Canada / Aquatic Contaminants Research Division; J.P. Smol, Queen's University / Department of Biology. Assessments of the environmental impacts of the Athabasca oil sands development in northeastern Alberta have noted the lack of long-term data and very limited information on pre-impact conditions. In this study we have used paleolimnological techniques to reconstruct long-term dynamics of PAH loadings, aquatic primary production, and zooplankton (Cladocera) assemblages over at least the last 50 years. We analysed dated sediment cores from 5 lakes within 35 km of the bitumen upgrading facilities in the center of the development area north of Ft McMurray, AB, and one reference lake, 100 km northwest of the upgraders. These lakes were undisturbed and received only atmospheric inputs. PAHs (47 analytes) increased precipitously beginning at the early 1970s, particularly C1-C4-alkylated PAHs and C1-C4-dibenzothiophenes (DBTs). In 4 of 5 near-field lakes, maximum concentrations were observed from the most recent (~2009-10) sediments. Total ( $\Sigma$ )PAH fluxes were 2.5 to 23-times greater than pre-1960 background levels. Increases were also evident in Namur Lake, and in a lake 22 km southwest of the upgraders, but were less

pronounced (2-8-fold) and had subsurface maxima in sediments dated to 2006. C1-C4 alkylated PAHs and DBTs predominated in all sediment samples, representing 55 to 89% and 1.8 to 17% of the  $\Sigma$ PAHs, respectively. The predominance of these groups in snow and precipitation in the region has been reported previously. Coincident with increased PAHs, climate-induced shifts in aquatic primary production are also likely contributing to lake-ecosystem changes. A paleo-ecotoxicological assessment of *Daphnia* relative abundances indicates that this sentinel species was not yet negatively impacted by decades of increased PAHs and other likely associated contaminants (e.g. metals). Multiple environmental stressors, namely climate warming and industrial contaminants, acting collectively since the latter half of the 20th century are influencing lake-ecosystem functions and zooplankton assemblages in the oil sands region. Stressors have forced freshwaters toward new ecological states largely distinct from those of previous centuries of lake-ecosystem history.

**428 An Assessment of Hg Contamination in Lake Chapala, Mexico: Implications for Ecosystem Health** M. Mora, Texas A&M University / Wildlife and Fisheries Sciences; Z. Torres, Texas A&M University / WFSC; R. Taylor, Texas A&M University / Veterinary Integrative Biosciences; D. Alvarez, Instituto Politecnico Nacional / CIIDIR; H. Buelna, Instituto Politecnico Nacional. The Lerma-Chapala Basin is the home to 10% of Mexico's human population. Lake Chapala is the largest tropical lake in Mexico and the ultimate receptor of a great variety of contaminants that are discharged through the basin to the Rio Lerma. In addition to being a major water source for the city of Guadalajara, Lake Chapala represents a major fishery and recreation resource for various communities surrounding the Lake, as well as for tourists from many parts of the country. Lake Chapala is one of the largest wintering areas for American white pelicans (*Pelecanus erythrorhynchos*), a species of special concern in the United States. We conducted a two year study to assess the impacts of Hg and other metals on fish and wildlife of Lake Chapala. Here we report the results of the analysis of Hg and MeHg concentrations in water, sediments, and three fish species which are part of the diet of fish-eating birds and also of many human inhabitants in the region. For the first year samples, mean Hg concentrations were  $0.015 \pm 0.002$  ng/ml in water,  $0.597 \pm 0.190$   $\mu$ g/g dw in sediments,  $0.626 \pm 0.065$   $\mu$ g/g dw in charal (*Chirostoma spp*),  $1.597 \pm 0.430$   $\mu$ g/g dw in carp (*Cyprinus carpio*), and  $0.176 \pm 0.113$   $\mu$ g/g dw in tilapia (*Oreochromis mozambique*). Carp from a nearby reservoir in San Antonio Guaracha, Michoacan had mean Hg levels of  $0.396 \pm 0.144$   $\mu$ g/g dw. The samples collected earlier this year are currently being analyzed. The results will be available for analysis and for further assessment of the potential negative effects of these Hg concentrations on fish-eating birds and humans. We will also present data on Hg in feathers from aquatic birds. This project contributes to one of the goals of the Commission for Environmental Cooperation regarding implementation of an action plan to eliminate sources of mercury pollution in North America. It also contributes to one of the seven priorities for action of the UNEP Global Mercury Partnership, which is to conduct research into mercury's atmospheric transport and fate.

**429 Ecosystem Response to Evolving Mercury Emissions** L. Levin, Electric Power Research Institute / Air Toxics Health & Risk Assessment; K. Vijayaraghavan, ENVIRON International Corp.. Both U.S. and Asian mercury emissions are rapidly evolving as economic and control steps are introduced in the two regions. A critical question is whether local and regional controls on point sources of mercury will result in significant changes in fish tissue levels of the substance as more distant sources increase their emissions. Repeated studies in a variety of lakes show that initially rapid changes in fish burdens of mercury are likely to be followed by slow adjustments to step changes in mercury depositing to waterways from the atmosphere. Aquatic fish populations act as band-pass filters for mercury deposition and methylation over time, serving as interannual transfer systems for methylmercury mass within the system. This study focuses on fish populations in a well-characterized lake in the northeastern U.S. and projects evolution in fish tissue levels of mercury following drops in U.S. utility emissions due to the Federal MATS rule, plus plausible emissions growth scenarios for Asian emissions. Both seasonal cycling of fish tissue mercury and longer term trends are dominated by one or both source categories depending on the growth projections for distant sources.

**430 Mercury in Fish on the California Coast: Indicators of Global**

**Atmospheric Contamination?** J.A. Davis, J. Ross, San Francisco Estuary Institute; G. Ichikawa, California Department of Fish and Game; W. Heim, Moss Landing Marine Laboratory; M. Stephenson, California Department of Fish and Game; K. Schiff, Southern California Coastal Water Research Project. The California State Water Resources Control Board's Surface Water Ambient Monitoring Program (SWAMP) has established a new statewide bioaccumulation monitoring program. The program began with a two-year screening survey of bioaccumulation in lakes and reservoirs in 2007 and 2008, followed by a two-year survey of the coast in 2009 and 2010, and a survey of rivers and streams in 2011. In the Coast Survey, 3,483 fish representing 46 species were collected from 68 locations. Five species were examined at each sampling location, including high trophic level species expected to accumulate relatively high concentrations of mercury. Many locations, 25 of the 68 sampled (37%), were in a high contamination category, with an average for the most contaminated species exceeding a threshold of 0.44 ppm used by the state in developing consumption advisories. Species with high concentrations were observed more frequently in the north and central coast regions than in the south, primarily due to regional differences in the suite of species sampled. The species with high concentrations were generally relatively old and at a high trophic position. The data from this survey indicate that methylmercury contamination is ubiquitous in the food webs of the California coast. Even remote location island locations were found to have a moderate degree of contamination. These data suggest that enough mercury is supplied to California coastal food webs to cause problematic bioaccumulation wherever long-lived predatory fish species are caught and consumed. The ubiquitous contamination observed in this survey, coupled with the minimal influence of local atmospheric sources in this region, suggests that atmospheric deposition of mercury from global sources may be a significant contributor to methylmercury in California coastal food webs. The contributions of other local terrestrial sources appear to be superimposed upon a background of global atmospheric deposition and other sources to coastal waters such as geological sources and the upwelling transport pathway. San Francisco Bay had relatively high concentrations and is an exceptional case where local sources, especially historic mercury mining, play a distinct role.

**431 Mercury biomagnification rate in aquatic food webs worldwide**

R.A. Lavoie, Queen's University / Biology; K. Kidd, Canadian Rivers Institute, University of New Brunswick / Biology, University of New Brunswick / Biology department; T. Jardine, University of Saskatchewan / Toxicology Center; M.M. Chumchal, Texas Christian University / Biology department; L. Campbell, Saint Mary's University / Biology. Trophic level is among the most important variables determining the overall concentration of mercury (Hg) in a given organism. The estimation of trophic level using nitrogen isotopes ( $\delta^{15}\text{N}$ ) revolutionized the study of Hg biomagnification. The overall rate of Hg biomagnification through food webs can be determined using the slope of  $\log(\text{Hg})$  versus  $\delta^{15}\text{N}$  regressions (called trophic magnification) and this parameter can be compared across systems that are chemically and biologically different. Mercury trophic magnification varies among aquatic food webs and to date there is no consensus on the main factors driving this variation. We compiled results from over 60 studies and 190 sites around the world (published and non-published) that measured Hg trophic magnification in aquatic food webs using  $\delta^{15}\text{N}$ . Studies ranged from freshwater to marine and from tropical to arctic ecosystems. Using GIS and statistical approaches, we examined whether variables of physico-chemistry (e.g., pH, percentage of wetland, DOC, productivity, Hg deposition, latitude and longitude) and community structure (species composition and food chain length) explained among-system differences in trophic magnification. Preliminary results suggest that trophic magnification was positively related to latitude and negatively related to mercury deposition. In contrast, Hg concentration at the base of the food chain was positively related to mercury deposition. Trophic magnification was lower in systems with longer food chain length. Specific spatial relationships will be presented. This study is among the first to comprehensively assess factors affecting biomagnification of mercury in diverse ecosystems worldwide.

**432 Ecogenetics – Can Epigenetic and Gene-Environment Interaction Studies Help Improve Mercury Risk Assessment?**

N. Basu, University of Michigan / Department of Environmental Health Sciences, University of Michigan / Department of Environmental Health; J. Goodrich, J. Head, University of Michigan. The risk assessment of mercury is complicated

due to innate inter-individual variation in exposure, biomarker values, and adverse outcomes. Recent findings by us and others suggest that epigenetic mechanisms and gene-environment interactions (which have seldom been included in mercury's risk assessment) may help explain some of this variation. In terms of epigenetics, studies on laboratory models (mink, chicken, perch) and wild animals (polar bears) reveals mercury-associated global DNA hypomethylation and impaired DNMT activity in some, but not all, organisms. Candidate gene studies in a cohort of dental professionals documents that methylation of SEPP is associated with significant changes in mercury biomarker levels. In terms of gene-environment interaction studies, work by us and others show that some genes that influence the absorption, distribution, metabolism and elimination (ADME) of mercury have polymorphic variants that can affect biomarker levels. For example, epidemiological research by our group has shown that polymorphisms in MT1A, MT1M, GSTP1, and SEPP1 can be associated with altered levels of urine and hair mercury. For both epigenetics and gene-environment interactions, high-throughput approaches are commercially available which we are now exploring in a range of studies. This presentation will provide a rationale by which ecogenetic approaches and information can be used to improve our ability to assess the human health and ecological health risks (both exposure and adverse outcomes) of mercury and help identify vulnerable groups.

**433 Mercury in Wetlands – A contribution to the definition of a global mercury policy**

J. Canario, IPMA IP / Aquatic Environment Department, IPMA I.P. / Aquatic Environment and Biodiversity, INRB IP/IPIMAR / Aquatic Environment and Biodiversity; C. Vale, IPMA I.P. The overall goal of the UNEP Global Mercury Partnership is to protect human health and the global environment from the release of mercury and its compounds by minimizing and, where feasible, ultimately eliminating global, anthropogenic mercury releases to air, water and land. Besides the decrease on mercury emissions in the northern hemisphere in the last decade, the amount of mercury circulating in the environment is still of great concern. This issue assumes a major problem in ecosystems, which due to their physical/chemical/biological characteristics, exchange mercury species mainly  $\text{Hg}^0$  (mercury volatile) and MeHg (methylmercury) with the atmosphere and food web, respectively. Wetlands, particularly salt-marshes, are among these key ecosystems where these Hg transformations and Hg escape occur. Mercury studies in Portuguese salt-marshes showed that salt-marsh plants and the surrounding rhizosphere environment are important areas of mercury retention and methylation. Levels of up to 30% of MeHg in salt-marsh sediments contrasting to the 0.01% of MeHg in non-vegetated ones and higher concentrations of both Hg and MeHg in root biomass poses a serious debate about whether salt-marshes are efficient for Hg phytoremediation. Additional studies in Portuguese salt-marsh plants in contaminated areas also showed that macrophytes are important sources of  $\text{Hg}^0$  to the atmosphere. Mercury vegetation-air fluxes measured in two salt-marsh plant species showed  $\text{Hg}^0$  emissions during three consecutive days, indicating that these ecosystems can act also as an important natural mercury source. The export nature of these ecosystems for mercury has been shown. These findings have serious implications on mercury pollution especially concerning mercury fate and speciation in wetland contaminated areas. Based on our work we believe that the definition of a global mercury policy must take into account wetland areas which occupied up to 9 million  $\text{km}^2$  of earth surface therefore incorporating these ecosystems in the UNEP monitoring and assessment mercury programs. Two types of measures should be envisaged. Encourage better understanding of mercury transformation and fluxes in key areas of the world, namely wetlands and areas where mercury is used in industrial processes. Improve technologies to reduce the mercury emissions and to sequester mercury in inorganic forms.

**434 Partitioning of total mercury and methylmercury between colloids and true solution in aqueous matrices**

D. Vignati, Irsa-Cnr, IRSA-CNR, UOS Brugherio, Université Paul Verlaine / LIEBE CNRS UMR 7146, Université Paul Verlaine / CNRS UMR 7146; S. Guedron, Université Grenoble 1 / ISTerre. Mercury is a priority pollutant which, upon conversion into the highly bioavailable and neurotoxic form monomethylmercury (MMHg), easily enters the food chain and can cause significant exposure in human beings; primarily via the consumption of contaminated fish and seafood. Bottom sediments and the associated interstitial waters are a privileged site for the inter-conversion of Hg into MMHg; while the partitioning of Hg and MMHg between colloids (particles smaller than 1  $\mu\text{m}$ , but larger than 1 nm) and true solution (the fraction smaller than 1 nm) can affect

their environmental fate and bioavailability. Because sediments constitute a critical environmental compartment for Hg and MMHg cycling, study of mercury partitioning at the sediment-water interface or in interstitial waters is a subject of particular scientific relevance. Some researchers already studied Hg/MMHg partitioning between colloids and true solution in marine waters and freshwaters; but, to our knowledge, analogous experiments for interstitial waters have not yet appeared in the scientific literature. Sediment cores and associated overlying waters (OW) were collected in Lake Geneva (Switzerland/France), Baihua reservoir (Guizhou Province, China) and in the Venice lagoon (Italy). OW were siphoned off the cores, filtered using Sterivex cartridges (0.45 µm nominal cut-off), subsampled for Hg and MMHg analysis and further fractionated by tangential flow ultrafiltration. Cores were sliced (under nitrogen atmosphere for anoxic waters/sediments) and the interstitial waters (IW) recovered by centrifugation. IW were then filtered and fractionated as for overlying waters. Both total Hg and MMHg concentrations were assayed by cold vapor atomic fluorescence spectrometry (CV-AFS). Concentrations of THg and MMHg were lower in Lake Geneva than in the Venice Lagoon and in the Baihua reservoir, reflecting the increasing contamination of the three studied sites. MMHg represented a very high percentage of THg (22–30 %) in the Baihua reservoir, while it never exceed 6 and 2 % in Venice lagoon and in Lake Geneva, respectively. Some significant correlations were found between total filterable and colloidal fractions for both total Hg and MMHg suggesting that some general mechanism of Hg partitioning probably exist.

**435 Albatross as Sentinels of Marine Pollution: Local and Global Factors** M.S. Bank, Harvard Medical School / Department of Environmental Health, Harvard School of Public Health / Dept of Environmental Health, Harvard Medical School / Harvard Center for Comparative Medicine, Harvard Medical School / Center for Animal Resources and Comparative Medicine; A.E. Vo, University of California, Berkeley / Museum of Vertebrate Zoology, Department of Integrative Biology; W. Sentman, Harvard University Extension School; S.V. Edwards, Harvard University / Museum of Comparative Zoology, Department of Organismic and Evolutionary Biology. Heavy metal pollution in the Pacific Ocean has garnered significant attention in recent years, especially with regard to rising mercury emissions from Asia. Uncertainty exists over the extent to which mercury in biota may have resulted from increases in anthropogenic emissions over time. Albatrosses, including those inhabiting the North Pacific, are wide-ranging, long-lived, keystone, avian predators. Consequently, they serve as ideal sentinel species for investigating the effects of historical and contemporary pollution as well as local and global factors related to heavy metal exposure, bioaccumulation, and ecotoxicological risk. To date, high levels of mercury and lead have been documented in albatross species throughout the Pacific. To address biotic exposure to these multiple stressors, here we synthesize and conduct meta-analyses of total mercury, methylmercury, and lead exposure data in Black-footed albatross (*Phoebastria nigripes*) and Laysan albatross (*Phoebastria immutabilis*). Our approach includes data from the field and literature, and for total mercury and methyl mercury, we use measurements from museum feathers spanning the past 130 years for Black-Footed albatross. We discuss the use and application of stable isotopes ( $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ ) as a way to control for temporal changes in trophic structure and diet and we demonstrate the importance of conducting speciation analyses for mercury to account for historical, curator-mediated, inorganic mercury contamination of specimens. Our data showed higher levels of inorganic mercury in older specimens of Black-Footed albatross as well as two non-pelagic species (control samples) lacking historical sources of bioavailable mercury exposure, which suggests that studies on bioaccumulation should measure methylmercury rather than total mercury when utilizing museum collections. Albatross species face heavy metal exposure threats at both local (lead) and global scales (mercury, and potentially heavy metals in plastics). Heavy metal toxicity along with other stressors may undermine current and future reproductive outcomes in these seabird species, although unraveling effects from specific metals in the context of a complex metal mixture presents some challenges. Collectively, our findings and review of the literature suggest that albatrosses in this region may be an effective marine flagship species and raising the profile of these organisms likely would successfully support broader biodiversity conservation efforts in the North Pacific.

**436 Losing bugs and birds: Investigating the impact of neonicotinoid insecticides on agricultural wetlands of Prairie Canada** C. Morrissey, University of Saskatchewan / Biology; A. Main, University of Saskatchewan

/ School of Environment and Sustainability; C. Michelson, University of Saskatchewan / Department of Biology; B. Clark, Environment Canada; A.J. Cessna, National Water Research Institute / Environment Canada, Environmental Health National Program / Agriculture and Agri-Food Canada; J. Hedley, Environment Canada; P. Mineau, National Wildlife Research Centre / Canadian Wildlife Service. Carson was the first to address the problems associated with the agriculture industry's "war on insects" and the haphazard use of insecticides. In the last 50 years, the development and use of insecticides has continued to rapidly expand in opposition with equally rapid pest resistance. A newer class of insecticides – neonicotinoids – though designed for improved agricultural production, are controversial because of their persistence and high leaching potential, and evidence in Europe and North America show that they are highly toxic to a wide range of non-target aquatic insects and other arthropods. Applications also directly overlap with peak seasonal invertebrate production and the avian breeding season for a range of species and evidence suggests populations of farmland and wetland birds are declining rapidly throughout North America and Europe and are potentially susceptible to neonicotinoids indirectly through loss of insect prey. In the Canadian Prairie Pothole region, intensive and widespread use of neonicotinoids particularly for canola seed treatments prompted us to investigate the fate and effects of these chemicals in agricultural wetlands. We are evaluating neonicotinoid concentrations in water and sediment in approximately 140 wetlands from April to September along with rapid wetland assessments to evaluate the landscape and ecological variables that are contributing to wetland contamination. We are further assessing seasonal changes in insect abundance in a subset of wetlands in areas with high and low neonicotinoid use in order to link observed changes to shifts in diet, growth and reproduction of local insectivorous bird populations. The outcomes of the study will lead to better informed risk assessment practices of neonicotinoids in addition to addressing the biological integrity of aquatic ecosystems in one of Canada's most ecologically and commercially productive agricultural environments.

**437 Occurrence of Current-Use Pesticides in Surface Water, Sediment, and Biological Tissue Across the USA** K.M. Kuivila, K.L. Smalling, M.L. Hladik, J. Orlando, U.S. Geological Survey. During the last two decades, studies by the U.S. Geological Survey have shown that numerous current-use pesticides are present in surface waters across the United States and occur both dissolved in the water and bound to sediments. Pesticide use is constantly changing, so analytical methods are frequently updated to analyze new pesticides in water and sediments. We design field studies to sample streams in small watersheds with specific use-settings, rather than to conduct widespread monitoring. One series of field studies was designed to focus on pyrethroid insecticides because of their increasing use and high toxicity to aquatic organisms. Due to their hydrophobicity, pyrethroids ( $\log K_{oc} > 5$ ) tend to bind to sediments. Of the 14 pyrethroids analyzed in small agricultural and urban streams across the country, 10 were detected in suspended and bed sediments. The most frequent detections were found in urban stream bed sediments, with bifenthrin being detected most frequently. In contrast, a wider variety of pyrethroids were detected in agriculturally influenced streams. Other field studies have focused on fungicides, a group of pesticides that is rarely monitored and whose registration and use have changed dramatically over the last 5 years. Fungicides are moderately hydrophobic ( $\log K_{oc}$  2 to 4) and are considered to be relatively persistent in water and sediments. Stream samples were collected from different agricultural areas across the country, including leafy greens, orchards, peanuts, potatoes, rice, and strawberries. Of the 37 fungicides analyzed, 20 were detected in water or sediments or both. Azoxystrobin, boscalid, myclobutanil, and pyraclostrobin were detected the most frequently and at the highest concentrations. Recently, methods have been developed to analyze tissue. Although most current-use insecticides and fungicides are only moderately lipophilic, they were detected in a variety of aquatic organisms, including amphibians, crabs and crab embryos, fish, and osprey eggs. Pyrethroids (bifenthrin, cyfluthrin, etofenprox), other insecticides (chlorpyrifos, fipronil, malathion), and fungicides (pyraclostrobin, tebuconazole) were detected in tissue samples from across the country. These results highlight the importance of updating analytical methods as pesticide use changes and analyzing multiple environmental compartments.

**438 Screening of pesticide in surface waters of four basins of the Pampas Region of Argentina with different degrees of agriculture intensities** D. Marino, Y. Elorriaga, Centro de Investigaciones del Medio Ambiente



(CIMA-CONICET), Facultad de Ciencias Exactas, Universidad Nacional de La Plata; V. Aparicio, Instituto Nacional de Tecnología Agropecuaria (INTA), Estación Experimental Agropecuaria Balcarce; G. Natale, P. Carriquiriborde, Centro de Investigaciones del Medio Ambiente (CIMA-CONICET), Facultad de Ciencias Exactas, Universidad Nacional de La Plata. The Pampas Region is the main agricultural district of Argentina predominantly by the alternated production of soybean and corn in summer and wheat in winter. Despite the wide spread of this activity little is known of its impact on surface water quality and the aquatic ecosystems at the regional scale. In this study, 70 pesticides were determined in surface water samples from 22 sites distributed along four agriculture basins, two mainly agricultural and two mostly extensive livestock. Samples were filtered through 0.45  $\mu\text{m}$  GF/F immediately after collection and transported in glass bottles to the laboratory. SPE extraction was performed with C18 OASIS HLB, within the 48h after collection. Pesticide analysis was performed by UPLC-MS/MS ESI(+/-) (Acquity-Quattro Premier). Spiking and isotopic dilution methods were used for quality assurance. A total of 35 samples were analyzed. Among all the pesticides measured, only 30 were positively detected. Conversely to the expected, patterns in the composition of detected pesticides were similar in both classes of basins, only four pesticides were not observed in the less agricultural basins. On the other hand, clear differences were observed in the abundance. Differences in the frequency of occurrence of some pesticides were observed when comparing summer and winter samplings (i.e. atrazine was 100% detected in summer and 90% in winter and metsulfuron methyl 60% in winter and not detected in summer). In addition, during the summer triazole fungicides and agricultural pyrethroids were detected in the 80% and 70% of the samples, respectively. Finally, imidazolinone herbicides were detected in the 20% of the cases. In the present study a broad screening of pesticides at regional scale was performed in the Argentinean Pampas, revealing for the first time the occurrence of a wide number of pesticides together with characteristic spatial and temporal patterns.

**439 Pesticide residues in prairie pothole sediments as influenced by three dominant land-use types** S. McMurtry, Oklahoma State University / Department of Zoology; J. Belden, L. Smith, D. Daniel, S. Morrison, Oklahoma State University; B. Tangen, R. Gleason, N. Euliss, U. S. Geological Survey. Wetlands throughout the Great Plains represent some of the most heavily impacted habitats in North America due to extensive cultivation of surrounding uplands. Indeed, these depressional wetlands are typically the terminus of the watershed and thus receive substantial runoff and sediment input from cultivated uplands, influencing multiple ecosystem services provided by wetlands. Pesticides, from application on adjacent cropland and from overspray, also have the potential to move into wetlands. The United States Department of Agriculture (USDA) offers various conservation programs (e.g., Conservation Reserve Program, CRP) designed to reduce soil erosion and runoff and to restore/protect natural resources influenced by agricultural activities. Although not designed to protect wetlands per se, conservation programs such as the CRP have a substantial influence on wetlands embedded within the restored grasslands. Therefore, this study was designed to assess the extent of pesticide residue in the three dominant land-use types (cropland, native prairie, and restored grasslands) surrounding depressional wetlands in the Prairie Pothole Region of North and South Dakota. Sediments were collected from 151 prairie pothole wetlands in North and South Dakota embedded in the three dominant land-use types; 49 cropland sites, 49 native prairie sites, and 53 restored grassland sites. Sediment samples were analyzed for glyphosate and organic carbon, and are currently being analyzed for triazine and chloroacetanilide herbicides, pyrethroids and organophosphate insecticides, and strobilurin fungicides. Mean ( $\pm$ SE) glyphosate concentrations were about four times greater ( $133.6 \pm 30.9$   $\mu\text{g/kg}$ ) in sediments of wetlands embedded in cropland catchments than in wetlands embedded in native prairie or restored catchments, which did not differ ( $31.5 \pm 5.7$  and  $36.9 \pm 10.7$   $\mu\text{g/kg}$ , respectively). In addition, 76% of samples from cropland sites had detectable concentrations of glyphosate compared to 55% and 51% for native prairie and restored wetlands, respectively. These results indicate that despite the presence of pesticides in non-cropland wetlands, concentrations are lower, and that restoration of previously cropped uplands improves pesticide residue status of embedded wetlands.

**440 The effects of a fungicide, miticide, and insecticide and possible synergy, on honey bee health and behavior** J.D. Johnson, University of Maryland / Dept of Toxicology, University of Maryland Baltimore / Med school, Dept. of Toxicology; J. Pettis, USDA/ARS Bee Research Lab. Pesticides enter hives by being carried in resources such as water, pollen, nectar, and propolis and by application by beekeepers to control mites and pathogens. In the matrices of wax, honey, and pollen stores, pesticides may partition, store, and potentially interact. Three pesticides that have been reported in bee hives were applied separately and together to observation hives. Fluvalinate, a pyrethroid miticide, was applied to empty comb. Chlorothalonil, a fungicide, was injected into pollen cells. Imidacloprid, a neonicotinoid, was applied as a sugar water spray onto in-hive bees. Emerged brood weights, pollen consumption, in hive census, foraging activity, and in-hive location mapping of 300 marked bees were monitored for one month. The hives were located in a row, ca. 1 meter apart and position was randomized for each trial. At the end of the month, the hives were dismantled, the equipment cleaned, a new colony established, and the hive dose and position re-randomized. Data from four replicates by month were collected. Significant drift was noted in the 3 pesticide and chlorothalonil treated hives even after accounting for row position effects, which were also significant. Effects on other behaviors and parameters measured will be discussed.

**441 Currently Used Pesticides in the Atmosphere in Agricultural Regions of Western Canada** R. Raina, University of Regina / Dept. of Chemistry & Biochemistry; N. Fergus, University of Regina / Department of Chemistry & Biochemistry; E. Smith, N. Al-Zaharni, University of Regina / Chemistry & Biochemistry; L. Sun, University of Regina / Department of Chemistry & Biochemistry; P. Hall, University of Regina / Department of Chemistry & Biochemistry. Initial studies on pesticides in the atmosphere of Western Canada began in 2003 with focus on the Lower Fraser Valley (LFV) agricultural region (berry crops), and the Canadian prairies (grains and oil seed crops). These studies have been further expanded in 2011 to include the Okanagan Valley of British Columbia where orchards and vineyards are present. Further sampling sites in each agricultural region were added to obtain additional spatial and temporal resolution to aid in assessment of regional and long-range transboundary atmospheric sources. These regions were selected due to the high usage of pesticides in western Canada, and differences in crop types, climate, and expected types and quantities of pesticides. In LFV we examined trihalomethyl thiofungicides, and organophorus pesticides (OPs), OP degradation products, and other selected herbicides and insecticides. For some pesticides atmospheric levels have exceeded  $10 \text{ ng/m}^3$  and more recent monitoring was used to assess whether atmospheric loadings have declines since 2006 when atmospheric levels indicated that pesticides such as captan, diazinon, and malathion continued to be favoured in the region. We will also investigate whether folpet which was replaced with chlorothalonil is still present in the atmosphere (since 2006). In the Okanagan Valley atmospheric concentrations of carbaryl also exceeded  $10 \text{ ng/m}^3$  in 2011 and were for the first time linked to its usage in apple thinning during the June drop, as well as on other orchard and vineyard crops throughout the growing season. In the prairies we also provided the first detection of azole fungicides in the atmosphere in the gas phase in 2010 during a year where many farmers experienced flooding conditions. Propiconazole was detected at maximum levels of  $78 \text{ pg/m}^3$  and has historical usage in the prairies as well as further south in the grain belt of the United States. A more recently recommended azole fungicide, prothioconazole, was also detected in the gas phase up to  $35 \text{ pg/m}^3$ . These two azoles showed levels below or near the method detection limit when sampling occurred during precipitation events even when temperatures were higher and was attributed to the higher water solubility of these fungicides. Long term-trends for some common high-usage herbicides (trifluralin, ethalfurin) and endosulfan in the Canadian prairies show annual shifts indicating the importance of climate and other pressures on farmers.

**442 California Department of Pesticide Regulation's Air Monitoring Network Results for 2011** E. Vidrio, P. Wofford, R. Segawa, California Department of Pesticide Regulation. The Department of Pesticide Regulation (DPR) is the public agency responsible for protecting California and its residents from adverse health effects from the use of pesticides. In February 2011, DPR implemented a long-term statewide air monitoring network (AMN) for measuring a total of 40 pesticides and pesticide breakdown products in three agricultural communities. As part of the monitoring

location selection process for the AMN, DPR evaluated and prioritized 226 communities in California as candidates for inclusion in the network. Salinas (Monterey County), Shafter (Kern County), and Ripon (San Joaquin County) were selected as the sampling locations for the air network. Four different samples are collected for analysis of the 40 pesticides; two different sorbent glass tubes, hand-packed Teflon® cartridges with sorbent media, and Summa canisters. 24-hour samples were collected every week at each of the three sites. The starting day varied each week with the actual dates being randomly selected. Results for the first calendar year indicate low levels of exposure. Thirty of the pesticides were detected, eleven of which had concentrations above the quantitation limit.

**443 Legacy organochlorine insecticides: What the Integrated Atmospheric Deposition Project can tell us** M. Venier, Indiana University / School of Public and Environmental Affairs, Indiana University; R.A. Hites, Indiana University / School of Public & Environmental Affairs,. The regulation of insecticides in the United States began with the banning of DDT in 1970 (as part of the Environmental Protection Act that also created the U. S. EPA) and has continued to the present with the restriction of lindane in 2009. More recently, another widely used insecticide, endosulfan (which has structural similarities to the now banned hexachlorocyclopentadiene family of compounds), has become suspect and is being phased out. The Integrated Atmospheric Deposition Network (IADN) has measured the atmospheric concentrations of several current use and legacy insecticides every 12 days at five locations near the North American Great Lakes since the early 1990s. Surprisingly, the DDT-related compounds are still present in the atmosphere, and their concentrations are decreasing only slowly. On the other hand, the atmospheric concentrations of the lindane-related compounds are decreasing rapidly. Despite the supposition that insecticides had been used only in agricultural applications, the atmospheric concentrations of most of these compounds track human population density. For example, the atmospheric concentrations of the chlordane-related compounds are higher in urban areas than in more remote agricultural areas. This presentation will focus on the spatial and temporal trends of the concentrations of these pesticides. Results will be put in the context of suggestions for the development of multi-national monitoring networks.

**444 Life cycle assessment for emerging technologies: The challenges of defining and applying impact metrics** T. McKone, University of California, Berkeley, University of California and Lawrence Berkeley National Laboratory, University of California, University of California and Lawrence Berkeley National Laboratory, University of California; C. Scown, Lawrence Berkeley National Laboratory / Environmental Energy Technologies Division; A. Horvath, University of California / Civil and Environmental Engineering. Emerging technologies--which we define as technologies in the research, development, or early commercialization stages--can pose a particular challenge to life-cycle assessment (LCA). In contrast to extant technologies, emerging technologies lack data on the materials, supply chains, processes, and performance of the technology in its intended application. With large-scale deployment of energy from biomass as a case study, we will identify and evaluate the challenges of defining and applying informative impact metrics for LCA. For many bioenergy LCAs overall impact is expressed in terms of carbon emissions or energy use. But an LCA must also address resource constraints, human and ecosystems health, water resources, technology options, and economic constraints. Confronting this broader set of metrics increases the challenges of making LCAs for emerging biofuels useful and relevant. We consider six challenges: (1) addressing multiple categories of impact (carbon, energy, health, water, social welfare, etc); (2) making comparisons and aggregating among different impact categories; (3) addressing spatial heterogeneity; (4) accounting for time in allocating impacts; (5) assessing transition paths as well as end states; and (6) confronting uncertainty and variability. Recognizing LCA as a process and not a product is key to meeting these challenges. Barriers to LCA arise because many stakeholders expect LCA to provide a clear and final answer. This serves only to highlight the flaws and uncertainties of LCA and fails to take advantage of the true power of LCA as an ongoing process that organizes both information and the process of prioritizing information needs. Addressing the world's need for near-term, cost-effective, and reliable production systems for bioenergy requires that the technological, social, economic, and environmental challenges be addressed in parallel.

**445 A spatially and temporally explicit life cycle inventory of air pollutants from liquid transportation fuels** C. Tessum; J. Marshall, University of Minnesota / Civil Engineering; J. Hill, University of Minnesota / Bio-products and Biosystems Engineering. The accuracy of life cycle assessment (LCA) in estimating environmental impacts is commonly limited by a lack of detail about when and where pollutants are released to the environment. We will present a spatially and temporally explicit life cycle inventory (LCI) for liquid transportation fuels built upon the existing GREET (Greenhouse Gases, Regulated Emissions, and Energy Use in Transportation) model from Argonne National Laboratory. GREET shows that ethanol fuel produced in the United States, as derived from either corn grain or corn stover, has higher life cycle emissions of most air pollutants than gasoline. Our results show that for ethanol these emissions are concentrated in the Midwestern "Corn Belt" of the United States and in rural areas. We find that increasing grid resolution improves the accuracy with which emissions are allocated to urban and rural areas. Temporal variations in life cycle emissions are most prominent in the agricultural stages of ethanol production. Our analysis also provides detailed chemical speciation of pollutants. For example, although cellulosic stover ethanol emits less fine particulate matter overall than corn grain ethanol, it emits a larger amount of black carbon. Altogether, the additional fuel LCI detail provided here will allow for air quality modeling in support of life cycle impact assessment (LCIA).

**446 Challenges of Life-cycle Assessment for Emerging Technologies** A. Horvath, University of California, Berkeley / Civil and Environmental Engineering, University of California, Berkeley / Civil & Environmental Eng.. Life-cycle assessment (LCA) is much practiced globally any more, but it faces significant challenges when analyzing products and services employing substantial components from emerging technologies. There is not much help available for LCA practitioners to overcome such problems, and this is a global problem. This presentation will summarize current thinking on these challenges, the reasons for their existence, and discuss a few product or service examples where LCA is struggling to assess emerging technologies. While this may have been a structural problem of LCA, I will discuss ways in which this could be minimized, if not eliminated, by concerted efforts made by the LCA community.

**447 Strategies for the LCA of emerging biomaterials** E. Bernier, National Research Council of Canada / Aquatic and Crop Resource Development Portfolio, National Research Council of Canada / Biotechnology Research Institute – Applied Ecotoxicology; C. Lavigne, National Research Council Canada / Aquatic and Crop Resource Development Portfolio; P. Robidoux, National Research Council Canada / Aquatic and Crop Resource Development Portfolio, National Research Council Canada / Project Leader. Bio-products and biomaterials are an important development axis at National Research Council Canada. LCA is a useful tool to confirm and promote their 'green' character, but also to raise potential issues and provide research guidance in order to avoid costly re-designs later. Three years of experience have allowed the development of strategies for assessing biomaterials-in-development, including: keeping the LCA study in-house for a better interaction with development scientists, thus improving research guidance opportunities; grasping the vocabulary of each industry/discipline (e.g. varying terminologies to describe bast fiber by-products and related processes); making reasonable assumptions on functional equivalence and insisting that the LCA results are conditional on their experimental verification later in project; making a careful analysis of scale-up issues when modeling lab-scale and pilot-scale processes; making an extensive use of the sensitivity analysis and using a color coding to display user-friendly results for a large number of scenarios; and putting some emphasis on ecotoxicological assessment and/or ecotoxicity screening in order to address potential emerging contaminants. In this presentation, these strategies are illustrated using sample results from three comparative LCA studies on biomaterials: polyisocyanurate insulating foams with and without kraft lignin, a by-product of wood pulping; phenol-formaldehyde resins with and without kraft lignin and carbon nanotubes; and polypropylene composites with glass, flax and hemp fibers. In general, these results show that using high-tech processes and additives in low-tech products in order to 'compatibilize' biomaterials is often unfavorable to the energy balance and overall footprint. For stationary biomaterial applications, the impacts in several categories are highly correlated to the transformation chain efficiency, while for automotive and aerospace applications, weight is the key parameter. Also, the end-of-life of biomaterials can be problematic in terms of carbon de-sequestration, methane from

anaerobic digestion, biological oxygen demand, and releasing mildly toxic but slowly degrading compounds in the aquatic environment (e.g. phenolic acids). Finally, allocation remains an issue depending on the extent to which raw biomaterials such as kraft black liquor or flax straw constitute 'waste'. Burden shifting to the 'land use' and 'aquatic eutrophication' categories may be unavoidable.

**448 Assessing sensitivity in consequential LCA using a long-term energy model: application to GHG emissions in the French energy sector** E. Bouvart, IFP Energies nouvelles / Economics and environmental evaluation department; S. Tchung-Ming, L. Patouillard, D. Lorne, F. Menten, IFP Energies nouvelles. LCA methodological principles such as defined in ISO standards are now widely applied to existing and future energy technologies. Many adaptations of these principles are possible and developed, paving the way for significantly different LCA approaches. When assessing new technologies, time horizon does not seem to be a distinctive factor to choose a given approach. However, the type of questions addressed is the right key to guide LCA practitioners in the definition of relevant methodology. This work especially pays attention to the definition of the goal and scope of the analysis, the appropriate functional unit and the results presentation in order to help for the clarification of the consequential LCA (CLCA) approach. This paper presents a case study aiming to tackle questions that could be raised by French Public Authorities (or industrials willing to comply with Public Authorities expectations) on GHG emissions impacts associated to the implementation of renewable fuel policies between 2020 and 2030. These policies intend to promote specific emerging technologies (2<sup>nd</sup> generation biofuels, transport electrification, etc.). For this purpose, a methodological framework is developed, based on CLCA, to include all processes of the French energy and transport sector that could be directly or indirectly affected by the considered decision. Calculations are performed using a long-term energy model based on cost minimization to both identify all "affected processes" based on market mechanisms and quantify corresponding GHG emissions trade-offs. This paper focuses on sensitivity analyses which are even more essential in the present prospective analysis (compared to ALCA applied on existing technology) due to additional uncertainties. Here, sensitivity analyses concentrate on energy policies that will apply in the mid-long run (renewable energy incorporation targets, GHG emissions reduction objective, fuel quality constraints etc.) and macroeconomic data such as energy resource prices. Scenario analyses are conducted to cover these issues. Results show that GHG emissions of the considered sectors can significantly vary according to energy policies that Public Authorities can set leading to different technological choices. In addition, a sectoral analysis allows to identify the substitution effects induced by relative price variations and their consequences on GHG emissions.

**449 Prospective LCA of next-generation manufacturing systems in the United States** E. Masaner, Northwestern University / Mechanical Engineering. Next-generation materials and manufacturing processes hold great promise for reducing societal energy use and environmental impacts. Examples include synthetic biology for scalable and low-impact manufacture of chemicals and fuels, advanced composites for lightweight vehicles, high-strength materials for more durable goods and structures, and nanomaterials for energy applications. Rigorous, prospective assessment of the potential environmental and economic implications of such technologies is critical for informing decisions on RD&D investments, policy incentives, and initial target markets, all of which can help usher advanced manufacturing technologies through the "valley of death." This presentation will discuss methodological issues and new structured analysis approaches for systems assessment of advanced manufacturing technologies in the United States from both life-cycle environmental and economic perspectives. LCA of manufacturing technologies is particularly challenging given that environmental and economic benefits are often accrued in other economic sectors (e.g., advanced composites will save energy in transportation, but not necessarily in manufacturing); thus, a consequential approach is crucial. Moreover, technology investments are such that major costs (and possibly environmental impacts) are often borne by the manufacturing sector while benefits are accrued in the end use sectors. This can create misalignment of environmental and economic incentives, which can stall technology development and deployment. The research presented here is designed to address these challenges. As background, we'll discuss a new mass-based input-output model that is being developed by the project team (Northwestern, NREL, and ORNL) to quantify the systems effects of materials and

process substitutions at the U.S. national level. Next, we'll discuss major modeling challenges for substituting traditional processes and materials with advanced processes and materials while also accounting for environmental and economic impacts in a consequential manner. We'll then discuss a new structured method for modeling technology change in the industrial sector and its affected end use sectors, which will provide the LCA community with a foundational approach for assessing advanced manufacturing systems at different time scales. We'll illustrate the research contributions with a U.S. case study.

**450 Anticipatory Life Cycle Assessment of Photovoltaics** B.A. Wender, Arizona State University / School of Sustainable Engineering and The Built Environment; T.P. Seager, Arizona State University / School of Sustainable Engineering & Built Environm. There is a critical need for life cycle assessment (LCA) during the formative stages of technology development, so that the systemic environmental consequences of new technologies may be identified and mitigated early in product development cycles. For example, several studies have called for the application of LCA to nanotechnology. However, LCA typically relies on detailed inventory and performance data collected from *existing* industries at commercial scales. In the case of nanotechnology, collecting manufacturing and use-phase LCA inventory data is problematic, both because nanotechnologies are proprietary and because the energy and material flows studied at the laboratory-scale will likely change as the technology matures. This necessitates the development of *anticipatory* LCA methods that can be used to explore potential environmental impacts of technologies and industries before they exist at scale, or as they evolve. Anticipatory LCA can be viewed as a quantitative scenario development tool used in situations of high uncertainty (e.g., nano-enabled energy technologies) to inform research, investment, and policy decisions. This requires a combination of laboratory-scale inventory measurements and technological performance modeling. This presentation demonstrates the value of, and challenges impeding, anticipatory LCA through a case study of photovoltaics (PV). While the economic experience curves in PV are well understood, and optimistically project the cost of PV to continuously come down as efficiency improves, it is not obvious that *environmental* experience curves are also monotonically declining, especially as PV increasingly incorporates nano-scaled materials. The results suggest that anticipatory LCA may uncover new research directions that will reduce life-cycle burdens, such as improving synthesis reactions yields, recovering catalysts from waste streams, recycling inert gases, and finding substitutes for scarce or critical materials. Thus, anticipatory LCA can result in reorientation of the research agenda within the laboratory towards pathways that reduce environmental impacts.

**451 Prospective Life-cycle Modeling of a CCS System Using Metal-Organic Frameworks for CO<sub>2</sub> Capture** R. Sathre, E. Masaner, Lawrence Berkeley National Laboratory. Metal-organic frameworks (MOFs) are promising new material media for carbon dioxide (CO<sub>2</sub>) capture. Their tunable adsorption patterns may allow efficient separation of gases, e.g. from power plant exhaust. Here we conduct scenario-based prospective life-cycle system modeling to estimate potentials and implications of large-scale MOF application for post-combustion CO<sub>2</sub> capture and storage (CCS). The methodological approach includes parametric system modeling to quantify relations between system components; scenario projections of plausible pathways for system scale-up; proxy data on analogous materials and processes; and uncertainty analysis of parameter significance. We estimate the system-wide primary energy use, greenhouse gas (GHG) emissions, metal resource use, and economic costs associated with projected large-scale MOF production and CCS deployment. We compare the performance of a MOF-based system to a currently more mature amine-based capture technology. We discuss balancing two critical factors: thermodynamic efficiency of the capture/regeneration cycle; and life-cycle embodied energy and cost of the material and its ancillary systems. We identify uncertain and/or variable parameters that strongly influence the performance of the MOF capture system, including solvent use, media lifespan, capture cycle time, capital cost, and regeneration energy. Optimization of one or more of these parameters may significantly reduce the GHG mitigation cost of MOF technology. This early-stage life-cycle modeling is intended to generate knowledge to inform decisions made by material scientists (e.g. performance targets for laboratory research), process engineers (e.g. critical design parameters for system scale-up), and policy makers (e.g. GHG mitigation potentials and costs of CCS).



**452 Consequences of our Plastic Age** C.M. Rochman, San Diego State University and University of California, Davis / Marine Ecology/Environmental Health. 8% of the world's fossilized fuels are consumed for the production of plastic products. Many of these items benefit society; however, increasing production, consumption and poor management of waste has led to large quantities of plastic debris in the environment. This has fueled concern among environmentalists, scientists, industry-workers and policy-makers about the safety of this material to our economy, health, wildlife and habitats. This presentation will introduce the session and will review our understanding of the costs and benefits of plastic to our economy and the well being of organisms. Using recent insights from field and laboratory experiments, I will discuss plastics as a multiple stressor in habitats taking into account the plastic material in addition to priority pollutants that sorb to them. To better understand hazards associated with plastics in aquatic habitats we examined (i) the fate of priority pollutants to the most abundant types of plastic debris in marine habitats and (ii) the toxicological consequences for fish that ingest this material. These findings will be discussed in relation to the need to integrate policies for managing waste, debris and other contaminants at international levels so that they reduce the problems caused by plastic debris that frequently crosses international boundaries.

**453 Comparing Polycyclic Aromatic Hydrocarbon (PAH) Profiles in Polystyrene Foam Before and After Deployment in San Diego Bay, CA** E. Hoh, San Diego State University / Graduate School of Public Health; T.N. Davis, San Diego State University; C.M. Rochman, San Diego State University and University of California, Davis / Marine Ecology/Environmental Health. In a previous study, we found that polystyrene (PS) foam recovered from San Diego County beaches contained greater concentrations of PAHs than other recovered plastic debris. This finding led us to further examine unexposed PS foam and PS virgin pre-production pellets. Both contained greater concentrations of PAHs than virgin polyethylene (PE), polypropylene (PP) and polyvinyl chloride (PVC). Furthermore PS foam contained much greater concentrations than the non-expanded virgin PS, suggesting that PAHs may be a byproduct of PS foam manufacturing. These results led us to examine PAH profiles in detail across different types of PS foam both exposed and unexposed to an urban bay. For three months, three replicates of each of three types of plastic foam (PS hard and soft white foam, and polyurethane foam) were deployed in San Diego Bay, CA. Another three replicates of each type were not exposed to the bay and were immediately stored at -20°C. We characterized and compared the chemical profiles among types of plastic foam and between those exposed to the bay using a non-targeted analysis using comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry (GCxGC/ToF-MS). This non-targeted analysis provides unbiased chemical analysis. Therefore, we were able to detect a broad array of chemicals including PAHs present in the plastic foams and determine changes of the chemical profiles of each type before and after deployment in the bay. Comparing the total ion chromatograms and 3D contour maps of the samples, a greater number of peaks were found in all three plastic foam types before deployment (PS foam hard: 1319, PS foam soft: 2105, and polyurethane foam: 4375) than those after deployment (PS foam hard: 930, PS foam soft: 1220, and polyurethane foam: 3091). Regardless of PS foam types, we observed a greater number of PAHs before deployment compared to after. Furthermore, our results suggest an increase of higher molecular weight PAHs from the bay but a loss of lower molecular weight PAHs. These results suggest that both PS foam and polyurethane foam leach chemicals into the marine environment as well as sorb contaminants from the environment. Further detailed data analysis including identification of individual peak is in process. Future results will be discussed with a goal of better risk assessment of plastic foam marine debris because our results suggest PS foam may be a source of PAHs to the marine environment.

**454 Detection of polybrominated diphenyl ethers (PBDEs) in tissue of seabirds and the ingested plastics** K. Tanaka, H. Takada, R. Yamashita, Tokyo University of Agriculture & Technology / Laboratory of Organic Geochemistry (LOG); Y. Watanuki, Hokkaido University / Faculty of Fisheries. Plastic debris has been frequently observed on sea surface even in open ocean. Ingestion of the plastics by marine organisms such as seabird has been often observed. It has been also revealed that marine plastics contain toxic chemicals derived from additives and sorption from ambient sea water. Transfer of the chemicals from the ingested plastics to internal system of marine organisms has been a question to be addressed. Several studies

focused on PCBs (polychlorinated biphenyls) to examine their transfer from ingested plastics to seabirds. So far only weak evidences of the transfer of chemicals from ingested plastics to seabird tissue have been obtained, thought they were significant. The reason behind the weak evidence was that PCBs are exposed to seabirds both through food web and the ingested plastics. The present study focused on polybrominated diphenyl ethers (PBDEs). PBDEs were measured in the abdominal adipose of 12 individuals of short-tailed shearwaters, *Puffinus tenuirostris*, that were accidentally caught during experimental fishing in the North Pacific Ocean. All the examined seabirds contained plastics in their digestive tract with 0.04 g to 0.62 g per individual. PBDEs were detected in all the seabird samples with concentration range of total PBDEs from 0.5 to 151 ng/g-wet tissue. In most of the individuals (8 of 12), lower brominated congeners (e.g., BDE#47, BDE#99, and BDE#100) were dominant. These congener profiles are similar to those commonly observed in pelagic fishes. In 3 individuals, however, BDE#209 was dominant over lower brominated congeners. Also in one individual, BDE#183 was dominant. BDE#209 and BDE#183 were major components of Deca-BDE and Octa-BDE technical products, respectively, and both are normally not detected in pelagic fish and, therefore, exposure of BDE#209 and BDE#183 to seabird through food web is unlikely. On the other hand, BDE#209 and BDE#183 were detected in the plastics in the digestive tracts of the seabirds examined in the present study. Furthermore, detection pattern of these congeners in the ingested plastics matched with that in the abdominal adipose, i.e., three individuals showed dominance of these higher brominated congeners both in ingested plastics and the adipose. These data suggest plastic-mediated transfer of chemicals to the internal tissue of marine organisms.

**455 Drawing Linkages from Presence of Marine Plastic Debris to Potential Pelagic Food Web Impacts** A. Cook, U.S. EPA. Plastic debris in the marine environment, including fragments and microplastics (pieces ranging in size from 0.3mm to 5 mm), contain organic contaminants that have either been added during the plastics manufacturing, or adsorbed from surrounding seawater. The pelagic food web (including food consumed by humans) is potentially contaminated as a result of these chemicals contained within or adsorbed to marine plastic debris. Research has shown that ambient persistent organic pollutants including polychlorinated biphenyls (PCBs) and dichlorodiphenyltrichloroethane and metabolites (DDTs) adsorb to plastic debris. Exposure to persistent organic pollutants (POPs) contained within the plastics or adsorbed onto them is likely to occur because marine organisms ingest the contaminated marine plastic debris. Few studies, however, have examined the effects of introduction of marine debris-borne contaminants on fish tissue. The prevalence of plastic debris in the marine environment poses the threat of transfer of POPs associated with this debris into the food web. Because POPs bioaccumulate in aquatic organisms that serve as prey for marine predators that are ultimately human food sources, humans may potentially be ingesting these bioaccumulated POPs. US EPA Region 9 and California EPA's Office of Environmental Health Hazard Assessment (OEHHHA) have partnered to complete a fish tissue study using fish from the family Myctophidae, which have a circumglobal distribution and perform key roles in ecological communities and the pelagic food web. These fish, taken from an area in the North Pacific referred to as the "Eastern Garbage Patch", have been sampled for the presence of POPs in fish tissue. Plasticizers and/or surfactants detected in fish tissue samples along with POPs can demonstrate likely plastic debris exposures that have the potential to bioaccumulate, thus potentially impacting the human food chain. Presence of POPs in fish tissue samples with no detections of plasticizers and/or surfactants does not allow the conclusion that plastic debris exposure is, at least partially, responsible for the presence of POPs. In either case, the potential for the POPs to bioaccumulate through the food chain still exists. Results of such studies will help resolve this potential problem and possible threat, and allow public policy and practices to evolve to address this issue.

**456 Ingestion and effects of microplastics on zooplankton** M. Cole, Plymouth Marine Laboratory; P. Lindeque, Plymouth Marine Laboratory / Zooplankton Group; E. Fileman, Plymouth Marine Laboratory; T.S. Galloway, University of Exeter / Biosciences Department. Small plastic detritus, or microplastics, are a widespread and ubiquitous contaminant of marine ecosystems. The ingestion of microplastics by a wide range of marine biota, including mussels, worms, fish and seabirds, has now been documented. However, despite the vital ecological role of zooplankton in the marine food web, the impact of microplastics on zooplankton has remained

under-researched. This work explores the uptake and biological effects of microplastics on a range of zooplankton species. We have used an integrated approach, combining feeding rate studies and novel bio-imaging techniques to document ingestion and egestion for a range of plankton displaying different feeding behaviours. Our initial experiments, using fluorescence microscopy and Coherent Anti-stokes Raman Spectroscopy (CARS), have established that many zooplankton species, including copepods and decapod larvae, can ingest a range of polystyrene microplastics (0.4 – 30 µm diameter). Using the copepod *Centropages typicus*, we have conducted exposures to natural assemblages of algae with and without 7 µm and 20 µm diameter microplastics. Our preliminary results indicate that the presence of 7 µm microplastics significantly decreases algal feeding. Suppressed feeding may have repercussions on zooplankton health (e.g. reduced egg production and growth), an area that will be the focus of our on-going research. We will present our latest progress with these studies with a view to understanding the impact that microplastics can have on zooplankton function and health.

**457 Exploring the physical effects of microscopic plastic particles in a sediment-dwelling marine polychaete** S. Wright, / Biosciences; T.S. Gallo-way, University of Exeter / Biosciences Department; A. Bakir, University of Plymouth, University of Plymouth Enterprise Ltd / Finance; R. Thompson, S. Rowland, University of Plymouth. Microplastics form a ubiquitous component of marine debris, accumulating in oceans and sediments worldwide from low densities to localized 'hotspots'. Since they occupy the same size fraction as sediment grains, microplastics may be ingested by low trophic infauna adopting indiscriminate feeding strategies, the ecotoxicological costs of which remains unknown. The current work aims to determine the potential effects of microplastics on the behaviour and health of an ecologically important benthic model invertebrate species. The sediment-dwelling marine polychaete *Arenicola marina* is a widely distributed, OSPAR-approved species and forms an important component of marine food webs. *A. marina* has already shown the capacity to ingest microplastics. We are using an integrated approach combining sublethal toxicological measurements at the individual level with population level parameters and bio-imaging techniques to determine the uptake and fate of microplastics in vivo. Preliminary 21 day exposures to 3% unplasticised polyvinylchloride (uPVC, 212-250 µm) by weight have indicated sublethal effects, with inhibited casting and therefore feeding rates. Consequently, growth rates may be suppressed. We now hope to assess the potential population-level effects which may arise from inhibited casting rates using Darwinian fitness parameters. Future work will include *A. marina* exposures to uPVC and polyethylene (PE) of different shapes and sizes at concentrations based on reported environmental levels to determine the effective concentration (EC10) and associated integrated health impacts. The results will offer insight into the potential impacts of marine litter on fundamental physiological processes in this important component of the marine benthos.

**458 The NOAA Marine Debris Monitoring and Assessment Project: Current Efforts and Future Applications** S.M. Lippiatt, National Oceanic & Atmospheric Administration / Marine Debris Program, NOAA / IMSG; C. ARTHUR, NOAA / IMSG; N. WALLACE, NOAA. Scientific monitoring of marine debris (solid, persistent, man-made material) is necessary in order to understand the source, distribution, abundance, movement, and impact of debris on local, regional, and global scales. Furthermore, establishing baseline information on the abundance and types of marine debris is necessary for evaluating the effectiveness of measures to prevent marine debris and its impacts. As a result of the potential for Japan tsunami debris to reach U.S. shorelines, the NOAA Marine Debris Program (MDP) has worked with various partners at sites along the West Coast, Hawaii, and Alaska to coordinate shoreline monitoring on a monthly basis. At all sites, surveys follow the established NOAA MDP shoreline survey protocols. Preliminary results will be shared and the applicability of monitoring information to mitigation and prevention efforts will be discussed.

**459 Being SMART about plastic debris: you can't manage what you don't measure** M. Browne, National Center for Ecological Analysis and Synthesis / University of California. In 2011, 47 companies that produce plastic signed a joint declaration to develop solutions to prevent plastic debris from contaminating habitats. This strategy calls for "(i) public-private partnerships aimed at preventing debris, (ii) working with the scientific community to better understand the scope, origins and impact of marine litter and the range of solutions to the problem, (iii) promoting comprehensive

science-based policies and enforcement of existing laws to prevent marine litter, (iv) promoting best practices in waste management particularly in coastal regions, (v) enhancing opportunities to recover plastic products for recycling and energy recovery, (vi) stewarding the transport and distribution of plastic resin pellets and products to its consumers and promote this practice along the supply chain". This talk will critically evaluate this strategy and some of the obstacles it may face. I will assess whether or not policy is up-to-date with current scientific understanding and whether "energy-recovery" is helpful. I will appraise the methods scientists and managers use to understand the problems of plastic debris and whether or not they are finding solutions. A key component of my talk will be discussing the cost-effectiveness of collecting data with and without hypotheses and adequate sampling designs. I will propose a new model for managing plastic debris that treats actions by regulators, managers and industry as large-scale field experiments. Through this ecoLOGICAL approach, the problem (hypothesis) is clearly articulated and the relevant sampling design and statistical framework chosen to determine whether the problem was fixed by the action. The advantage of this approach, over existing models, is that it provides a specific, measurable, achievable, realistic and time-scaled approach to measure the progress of industry (as well as scientists, government and public) is making toward fulfilling its aim in reducing plastic debris.

**460 Alternatives to the Fish Early Life-Stage Test: A Research Strategy for Discovering and Annotating Adverse Outcome Pathways During Fish Development** D.C. Volz, University of South Carolina, University of South Carolina / Department of Environmental Health Sciences; D. Villeneuve, U.S. Environmental Protection Agency; H. Aladjov, International QSAR Foundation; G. Ankley, U.S. Environmental Protection Agency; S. Belanger, The Procter & Gamble Company; K. Crofton, U.S. Environmental Protection Agency; M. Embry, ILSI Health and Environmental Sciences Institute; D. Hinton, Duke University; M. Hornung, U.S. Environmental Protection Agency; T. Hutchinson, Centre for Environment, Fisheries, and Aquaculture Science; T. Iguchi, National Institute for Basic Biology; R. Johnson, U.S. Environmental Protection Agency; M. Leonard, L'Oréal; D. Mount, T. Norberg-King, U.S. Environmental Protection Agency; L. Ortego, Bayer CropScience; S. Padilla, U.S. Environmental Protection Agency; R. Tanguay, Oregon State University; J. Tietge, U.S. Environmental Protection Agency; L. Truong, Oregon State University; G. Veith, International QSAR Foundation; L. Wehmas, Oregon State University; G. Whale, Shell Health. The OECD 210 fish early life-stage (FELS) test is the primary guideline test used to estimate chronic fish toxicity, as well as support ecological risk assessments and chemical management programs around the world. As a step toward developing alternatives to the FELS test, a HESI-sponsored expert workshop was convened in May 2012 to continue discussing approaches for screening and prioritizing chemicals for FELS testing. These discussions were built on the tiered testing strategy initially proposed by Volz et al. (2011). While this strategy was first illustrated using three Adverse Outcome Pathways (AOPs) relevant to early fish development, we recognized that the initial screening tier must be expanded to a broad range, or battery, of toxicologically relevant AOPs to account for multiple mechanisms of toxicity within and across chemical classes. Therefore, the primary objective of the May 2012 workshop was to begin identifying and discussing the scope and breadth of potential AOPs during early fish development. Based on discussions prior to and during the workshop, we concluded that, while providing a strong foundation to begin defining AOPs during early fish development, the existing peer-reviewed literature is likely not sufficient for establishing quantitative linkages across multiple levels of biological organization. Therefore, we outlined a comprehensive research strategy to (1) systematically discover, characterize, and annotate AOPs during early fish development and (2) prioritize development of these AOPs based on current restrictions and demands to reduce animal use for toxicity testing, particularly in the European Union. The overall goal of this research strategy is to provide the conceptual and scientific foundation for identification and development of resource-efficient predictive assays that address the toxicological domain of the OECD 210 test. This presentation will provide an overview of key findings from the workshop, as well as a summary of our proposed approach for discovering and annotating AOPs during early fish development. The contents of this abstract neither constitute nor reflect official US EPA policy.



**461 Discovering and Annotating Fish Early Life-Stage (FELS) Adverse Outcome Pathways: Putting the Research Strategy into Practice** D.L. Villeneuve, U.S. EPA / National Health and Environmental Effects Research Laboratory; D.C. Volz, University of South Carolina / Department of Environmental Health Sciences; H.T. Aladjov, Bulgarian Academy of Sciences / IBPhBME; G.T. Ankley, US EPA / Office of Research and Development, National Health and Environmental Effects Research Laboratory, Mid-Continent Ecology Division; S.E. Belanger, The Procter & Gamble Company / Environmental Stewardship Organization, The Procter & Gamble Company / Central Product Safety Department; K.M. Crofton, ORD, US EPA / Integrated Systems Toxicology Division, NHEERL; M.R. Embry, ILSI Health & Environmental Sciences Institute / Senior Scientific Program Manager; D.E. Hinton, Duke University / Nicholas School of the Environment; M.W. Horning, U.S. EPA / National Health and Environmental Effects Research Laboratory / Mid-Continent Ecology Division; T. Hutchinson, Centre for Environment, Fisheries, and Aquaculture Sciences (CEFAS); T. Iguchi, National Institute for Basic Biology; R. Johnson, U.S. Environmental Protection Agency / ORD/NHEERL/MED; M. Leonard, LOreal; D.R. Mount, US Environmental Protection Agency / ORD; T.J. Norberg-King, U.S. EPA / ORD, NHEERL, Mid-Continent Ecology Division; L.S. Ortego, Bayer CropScience / Ecotoxicology; S. Padilla, U.S. Environmental Protection Agency / Neurotox; T. Robert, Oregon State University; J.E. Tietge, US EPA / Office of Research and Development, National Health and Environmental Effects Research Laboratory, Mid-Continent Ecology Division; L. Truong, Oregon State University / Department of Environmental and Molecular Toxicology, Environmental Health Sciences Center, The Oregon Nanoscience and Microtechnologies Institute and the Safer Nanomaterials and Nanomanufacturing Initiative; G. Veith, International QSAR Foundation; L.C. Wehmas, Oregon St. University / Mid-Continent Ecology Division; G. Whale, Shell Health, Research and Technology Centre. In May 2012, a HESI-sponsored expert workshop yielded a proposed research strategy for systematically discovering, characterizing, and annotating fish early life-stage (FELS) adverse outcome pathways (AOPs) as well as prioritizing AOP development in light of current restrictions and calls for reduction in the use of animals in testing, particularly in the European Union. This presentation illustrates that strategy using specific examples. The scope of AOP development was defined by the desire to develop alternatives to the OECD 210 FELS toxicity test which could increase efficiency and reduce cost. Key anatomical and physiological events occurring during the developmental period covered by the FELS test were identified. Review of the extant peer-reviewed literature identified existing knowledge concerning the normal regulation of those developmental events and associated physiological functions. Using the key event of swim bladder development and inflation as an example, we illustrate how basic biological knowledge was mined to develop a series of putative AOPs applicable to FELS development. One set of putative AOPs focused on disruptions to swim bladder development while another focused on disruptions to swim bladder function. These putative swim bladder-specific AOPs can be used to identify potential molecular screening assays that may be predictive of impaired swim bladder development or inflation or additional endpoints for inclusion in an extended fish embryo test, as well as to identify critical research gaps. Finally, we discuss the relative priority of conducting research to develop and fill gaps in swim bladder-related AOPs in the context of the overall strategy. The contents of this abstract neither constitute nor reflect official US EPA policy.

**462 Development and validation of OECD test guidelines on mollusc reproductive toxicity tests** V. Ducrot, INRA / Ecotox & Quality of Aquatic Ecosyst, INRA / Ecotoxicology and Quality of Aquatic Ecosystems; A. Sieratowicz, Johann Wolfgang Goethe University; L. Lagadic, INRA / Ecotox & Quality of Aquatic Ecosyst; J. Oehlmann, Johann Wolfgang Goethe University; D. Azam, INRA; R. Brown, AstraZeneca; M. Coke, M. Collinet, INRA; J. Dobrick, P. Egeler, ECT Oekotoxikologie GmbH; H. Holbech, University of Southern Denmark; T. Hutchinson, Cefas; A. Jach, BASF AG; K. Kinnberg, University of Southern Denmark; G. Le Page, AstraZeneca; P. Lorenz, ECT Oekotoxikologie GmbH; I. Planojevic, Universiteit Antwerpen; K. Ruppert, Johann Wolfgang Goethe University; C. Schmitt, Universiteit Antwerpen; U. Schulte-Oehlmann, Johann Wolfgang Goethe University; C. Veauvy, C. Askem, A. Smith, Cefas; L. Weltje, BASF AG / Agricultural Centre, BASF SE / Crop Protection; P. Matthiesen, Centre for Ecology and Hydrology / Far Sawrey. To date validated guidelines in line with the OECD Conceptual Framework for the Testing and Assessment of Endocrine Disrupting Chemicals have been developed

for rodents, amphibians, fish, insects and crustaceans. Tests for vertebrates are well represented in the battery but, except for arthropods, no further invertebrate group has been considered. The comparison of endpoints relevant for reproduction in invertebrates shows in many cases a much higher sensitivity in molluscs versus daphnids. Based on this result, the OECD test guideline programme has been extended in 2011 to cover reproduction effects of chemicals. Existing mollusc toxicity test protocols have already been critically reviewed and compared in the literature and in a OECD Detailed Review Paper on Mollusc Life-Cycle Toxicity Testing that identifies two relevant candidate species for developing such freshwater tests: *Potamopyrgus antipodarum* and *Lymnaea stagnalis*. However, this review did not clarify which toxicity test design and test conditions are the most appropriate for chemicals assessment with the selected species. Therefore, a guideline will be developed describing partial- and two generation full-life cycle test procedures in these species, so as to propose a balanced suite of apical mollusc toxicity tests applicable for the assessment of any type of chemical, including endocrine disruptors, as level 5 assays as described in the OECD Conceptual Framework. The guideline project is led by a consortium of European experts on mollusc toxicity tests (Germany/United-Kingdom/France/Denmark) from academia, industry and stakeholders. To date, expert knowledge has been gathered and formed the basis of draft standard operating procedures (SOPs) for the culture and test implementation in both species. Pre-validation tests have been performed involving 10 partner labs. They consisted in a 28-d study with Cadmium (Cd) and Bisphenol A (BPA) in *P. antipodarum* and in a 56-d study with Cd and tributyltin (TBT) in *L. stagnalis*, cumulative individual fecundity being used as the main endpoint. Pre-validation with Cd and TBT were successful in both species but results of the BPA tests showed higher variability due to exposure issues. Current work is focusing on the optimization of culture and reproduction test methods for e.g. the assessment of reproduction enhancing compounds. Obtained results and draft SOPs will be submitted to VMG-Eco in November 2012 for validation, before a ring test is conducted.

**463 Results of the OECD validation study of the Zebrafish Embryo Toxicity Test and Relationships to Acute Fish Toxicity Tests** S.E. Belanger, The Procter & Gamble Company / Environmental Stewardship Organization, The Procter & Gamble Company / Central Product Safety Department; T. Braunbeck, University of Heidelberg / Department of Zoology; F. Busquet, ALTERTOX; G. Carr, The Procter & Gamble Company / Quantitative Sciences; A. Gourmelon, OECD / Environment, Health and Safety Division; M. Halder, European Commission / DG Joint Research Centre, IHCP, EURL ECVAM; A. Lillicrap, Norwegian Institute for Water Research; J. Rawlings, The Procter & Gamble Company / Environmental Stewardship Organization; R. Strecker, University of Heidelberg / Department of Zoology; S. Walter-Rohde, UBA, German Federal Environment Agency. The OECD Acute Fish Toxicity Test Guideline (TG 203) is an integral component in the environmental safety assessment of chemicals globally. A promising alternative approach to the acute fish toxicity test is based on the use of zebrafish embryos. In 2005, the German Federal Environment Agency submitted a draft TG on "Fish embryo toxicity (FET) test" to the OECD TG Program and a supportive Background Paper. OECD established an *ad hoc Expert Group on the FET* that concluded a validation study should be performed. The validation study evaluated the transferability, and the intra/interlaboratory reproducibility of the Zebrafish FET (ZFET). Newly fertilized zebrafish eggs were exposed for up to 96h to chemicals. Four apical endpoints are recorded daily as indicators of acute lethality in fish: coagulation of the egg, lack of somite formation, non-detachment of the tail bud from the yolk sac and lack of heart-beat from which LC50 values were determined. Twenty chemicals of various modes of action and physical-chemistry profiles were tested at 5 different concentrations in 3 independent runs in at least 3 laboratories with appropriate controls. Stock solutions and test concentrations were analytically confirmed for 11 chemicals. The 96-h LC50s for the suite of compounds ranged from 0.028-54,800 mg/L. Intralaboratory variability was less than interlaboratory variability for most chemicals. Interlaboratory variability was highest for the most toxic compounds or for chemicals that possessed challenging physical-chemical profiles. The *ad hoc Expert Group* concluded results were acceptable and consistent with expectations for ecotoxicity testing. A comparison of FET toxicity data and corresponding fish acute toxicity data (229 chemicals, 20 modes of action) was compiled to assess the overall relationships between the two. Regressions of FET versus fish acute toxicity were generated that resulted in a slope of 0.99, intercept of -0.19 and correlation



coefficient of 0.95 supporting a conclusion that the FET is a robust test method that predicts acute fish toxicity well. "Disclaimer: Opinions expressed and arguments employed herein are those of the authors and do not necessarily reflect the official views of the OECD or of the governments of its member countries, or the European Commission"

**464 Effectopedia: the collaborative science approach to adverse outcome pathways** H.T. Aladjov, Bulgarian Academy of Sciences / IBPhBME; G. Veith, International QSAR Foundation. The 21<sup>st</sup>-Century shift to a more prospective hazard identification and hypothesis generation requires strategic use of systems biology, QSAR and toxicological information. Adverse Outcome Pathways (AOPs) offer a unifying concept that can integrate the specialized knowledge from various disciplines and describe the causal linkages among biological responses over time. Effectopedia is an open-knowledge aggregation and collaboration tool for delineating AOPs in an encyclopedic manner with greater predictive power. It is designed to populate "AOP space" both with discrete cause-effect studies and with critical reviews in biology that are relevant to toxicology. To achieve both human and machine interpretability, Effectopedia uses an ontology-enhanced, natural language interface that offers clarifying questions and special tags to define the semantic knowledge while preserving the natural language description of the AOP's elements. The use of ontologies allows Effectopedia users the added advantage of publishing their contributions as nanopublications. Effectopedia serves as a visual editor to delineate of causal linkages at any level of biological organization and test species. It creates a common organizational space that (1) helps experts identify exactly where their specialized knowledge is needed in order to extend the causal linkages of biological responses and (2) creates a web-based conference room for dialogue and synthesis by experts with interest in a specific AOPs. Effectopedia creates live scientific documents that are instantly open for focused discussions and feedback, whilst giving credit to original authors and reviewers. Uncoupling the review and contribution processes allows different organizations to define their own set of criteria for quality assurance and associate them with the existing pathways while not slowing down the open (Wiki inspired) stream of contributions. New evidence of case studies, AOPs or discrete AOP segments can be distributed to interested contributors immediately, keeping Effectopedia's information current and providing historical documentation of its evolution.

**465 Adverse Outcome Pathways During Zebrafish Embryogenesis: A Case Study Using Paraoxon** K.L. Yozzo, University of South Carolina / Environmental Health Sciences; D.C. Volz, University of South Carolina, University of South Carolina / Department of Environmental Health Sciences. Using paraoxon as a reference acetylcholinesterase (AChE) inhibitor, the objective of this study was to develop an Adverse Outcome Pathway (AOP) that provided quantitative linkages across multiple levels of biological organization during zebrafish embryogenesis. Our hypothesis was that, similar to chlorpyrifos, developmental exposure to paraoxon (the neurotoxic metabolite of parathion) from 5 to 96 hours post-fertilization (hpf) results in concentration-dependent inhibition of AChE activity, leading to secondary motoneuron abnormalities and altered locomotion at 96 hpf. Within normal embryos, we first demonstrated that *ache* transcripts and AChE activity increased in a stage-dependent manner following segmentation. We then showed that static exposure of embryos to paraoxon (31.2-500 nM) from 5 to 96 hpf resulted in significant stage- and concentration-dependent inhibition of AChE activity, albeit these effects were fully reversible within 48 h following transfer to clean embryo media. Surprisingly, even in the presence of significant AChE inhibition, exposure to non-teratogenic paraoxon concentrations ( $\leq 250$  nM) did not adversely impact secondary motoneuron morphology nor larval locomotion based on confocal microscopy and preliminary behavioral evaluations at 96 hpf, respectively. Therefore, we investigated the potential effects of paraoxon exposure on spontaneous tail contractions at 26 hpf – an early locomotor behavior that (1) results from innervation of primary (not secondary) motoneuron axons to target axial muscles and (2) occurs in the absence of visual cues and detectable AChE activity. Interestingly, we found that, compared to vehicle controls, the frequency of spontaneous tail contractions was significantly higher following exposure to paraoxon concentrations as low as 31.2 nM (the lowest concentration tested), an effect that was not blocked by co-exposure with a non-selective muscarinic nor nicotinic acetylcholine receptor (AChR) antagonist. Overall, our data suggest that (1) normal AChE activity is not required for embryonic development and (2) AChE-independent tail

contractions at 26 hpf are sensitive to paraoxon exposure, an effect that is likely not due to direct paraoxon binding to AChRs. Using a well-studied reference chemical, this study highlights the potential challenges in developing AOPs that provide quantitative linkages from molecular initiating events to endpoints relevant for ecological risk assessments.

**466 Alternative strategies for assessing effluent toxicity in fish: A comparison of the fish embryo test and the larval growth and survival test** M.K. Sellin Jeffries, Miami University / Department of Zoology, Miami University; A.E. Stultz, Miami University / Department of Zoology; J. Rawlings, S. Belanger, Procter & Gamble; J.T. Oris, Miami University / Department of Zoology. In the United States (US), any facility discharging effluent directly to surface waters must conduct routine evaluations of whole effluent toxicity (WET). One method commonly used to obtain WET data is the 7 day larval growth and survival test (LGS) in which newly hatched fathead minnows (FHM), *Pimephales promelas* are exposed to effluents for 7 days with mortality as an endpoint. Given the increasing emphasis on animal welfare in toxicity testing, the goal of the current study was to identify an alternative test method capable of fulfilling the regulatory requirements for WET data, while minimizing the use of protected organisms or life stages. One such alternative to the LGS is the fish embryo toxicity test (FET), which has been sanctioned for WET testing in the European Union using embryonic zebrafish (ZF, *Danio rerio*). In order for the FET to be adopted in the United States, it would ideally be applicable to species commonly used in contract laboratories (i.e., FHMs) and demonstrate comparable sensitivity to the LGS. Thus, the main objectives of this study were 1) to develop test methods that harmonize the FHM-FET with the ZF-FET and the ZF-LGS with the FHM-LGS and 2) to compare the sensitivity of the FET and the LGS using both FHMs and ZF. To accomplish the first objective, tests using existing protocols for the FET and LGS were carried out with both species under different temperature and feeding regimes in order to determine optimal test conditions. The existing protocols were then modified and applied to the remaining experiments. To address the second objective, lethal and sublethal (e.g., length, weight, otolith size, deformity frequency) endpoints were evaluated following FET and LGS testing of each species using the reference toxicant 3,4-dichloroaniline and two types of effluents. These results provide evidence indicating the ability of the FHM-FET to serve as an alternative to the FHM-LGS for WET testing carried out in the US.

**467 Yet another analysis of sample size effects on the estimation of LC50 in common toxicity experiments, and free software to analyze your data!** G. Carr, The Procter & Gamble Company / Quantitative Sciences GCO; J. Rawlings, J. Brill, The Procter & Gamble Company / Environmental Stewardship Organization; S.E. Belanger, The Procter & Gamble Company / Environmental Stewardship Organization, The Procter & Gamble Company / Central Product Safety Department. Statistical methods for concentration-response models of quantal responses such as the probit model, are widely known and applied. Recent work on the design of these experiments has focused on substantial reductions of animal usage. A clear understanding of the tradeoffs to implementing these reductions is not readily available, however. To close this gap, extensive computer simulations were performed to quantify the effects of sample size on the quality of statistical results. The placement of exposures relative to the true, but unknown, LC<sub>50</sub>; the slope of the response; and the group sizes, must all affect the properties of the estimator. The results obtained in these simulations reflect, and quantify, the intuition that reducing study size must negatively impact the quality of results. The likelihood of a successful study, when group sizes are pushed to minimum levels, is shown to be highly dependent on the true, but unknown, LC<sub>50</sub> being well centered in the range of concentrations tested. When test concentrations are not well centered on the true LC<sub>50</sub>, the performance of the method is greatly degraded. In the course of our work on the development and validation of the fish embryo test for acute toxicity, a program was developed to fit probit-type models with a special class of confidence interval for the LC<sub>50</sub> (profile likelihood) that is more computationally difficult, but far superior to the standard interval method in problematic cases. Such cases are more likely to occur in smaller sized studies. A freely available program to fit this class of models in the R language for statistics is also presented, which includes the more robust confidence interval calculations and high-quality graphical summaries.

**468 Contaminants of Emerging Concern Early Warning Network: Regional Expansion of National Centers for Coastal Ocean Science Mussel Watch Program** K. Kimbrough, G. Lauenstein, NOAA/National Centers for Coastal Ocean Science / NCCOS Mussel Watch Program; J.D. Christensen, National Oceanic and Atmospheric Administration / Coral Reef Conservation Program, NOAA Ocean Service; N. Dodder, SCCWRP; K.A. Maruya, Southern California Coastal Water Research Project, SCCWRP; S. Weisberg, Southern California Coastal Water Research Project; D.A. Alvarez, USGS Columbia Environmental Research Center; E.T. Furlong, U.S. Geological Survey / National Water Quality Laboratory; S. Klosterhaus, San Francisco Estuary Institute; D. Gregorio, California Water Resources Control Board. A California pilot project was launched in 2009 to determine which, if any, contaminants of emerging concern (CECs) should be added to future Mussel Watch (MW) Program monitoring activities. The objectives of this study were to: 1) maintain connection to long-term MW by sampling a subset of historical sites for legacy and metal constituents; 2) expand spatial coverage to provide stratification by land use (urban, low development and agricultural) and proximity to regulated discharges; 3) focus analytical resources on high priority CECs (e.g. PPCPs, household chemicals and current use pesticides); 4) examine the efficacy of passive sampling devices (PSDs) as surrogates for contaminant exposure and bioaccumulation; and 5) create a regional-state-federal partnership to more efficiently and comprehensively utilize monitoring resources. Mussels (native and caged) were collected from 68 stations (25 historic) during 2009-10 and analyzed for more than 200 individual CECs; a subset of these samples were also analyzed for alternative flame retardants, perfluorinated chemicals and carbon nanomaterials. PSD arrays including Polar Organic Chemical Integrative Samplers (POCIS), solid phase microextraction (SPME) and polyethylene devices (PEDs) were deployed and retrieved at 11 sites for comparison with tissue CEC residues. The resulting data was analyzed to characterize the extent and severity of tissue CECs; assess for differences by land use and discharge categories; and investigate for concordance between tissue and PSD concentrations. This pioneering study will help guide future CEC monitoring efforts at the regional, state and federal levels.

**469 Occurrence of CECs in Bivalve Tissues from the California Mussel Watch Pilot Study** N. Dodder, SCCWRP; S. Klosterhaus, San Francisco Estuary Institute; R. Grace, Axyx Analytical Services; J. Ramirez, B&B Laboratories; M.J. La Guardia, Virginia Institute of Marine Science / Environmental & Aquatic Animal Health, Virginia Institute of Marine Science / Environmental Science; L. Ferguson, Duke University / Department of Civil and Environmental Engineering, Duke University / Nicholas School of the Environment, Duke University / Department of Civil & Environmental Engineering, Pratt School of Engineering / Department of Civil & Environmental Engineering; K. Kimbrough, G. Lauenstein, NOAA/National Centers for Coastal Ocean Science / NCCOS Mussel Watch Program; D. Gregorio, State Water Resources Control Board – CA; K.A. Maruya, Southern California Coastal Water Research Project, SCCWRP. In 2009, a pilot project was initiated within NOAA's Mussel Watch Program to monitor for contaminants of emerging concern (CECs) in California coastal waters. Native bivalves were collected from 68 stations extending the length of California's coastline. Stations were categorized by land use and proximity to regulated discharges. Approximately 150 CECs were measured by multiple laboratories, using primarily GC-MS and LC-MS/MS based methods. The CECs detected were (number measured/number detected): pharmaceuticals and personal care products (PPCP) (89/31), polybrominated diphenyl ethers (PBDE) (26/16), current use pesticides (CUP) (26/7), perfluorinated compounds (PFC) (12/5), hexabromocyclododecane (HBCD) and alternate flame retardants (9/4), and alkylphenols (AP) (4/4). The classes had sum concentrations per station of PPCP = AP > PBDE > CUP = HBCD = PFC. Single walled carbon nanotubes were not detected. CECs were highest for stations classified as urban, with the exception of CUPs which had higher concentrations for agriculturally-influenced stations. The CEC profiles (types and abundance) for urban stations were consistent across northern and southern population centers within California, indicating a generic rather than regional management approach is warranted. The influence of storm water and wastewater treatment plant discharges was also investigated. These results will help suggest CECs for inclusion in future Mussel Watch surveys and California-specific monitoring projects.

**470 Polyethylene-Water Partition Coefficients for In Situ Passive Sampling of Contaminants of Emerging Concern in Santa Monica Bay and Los Angeles Harbor** A.S. Joyce, University of Southern California / Chemistry, Univ. of Southern California; M.S. Pirogovsky, University of Southern California / Chemistry; W. Lao, Southern California Coastal Water Research Project; J.F. Haw, University of Southern California / Department of Chemistry; R.G. Adams, Loyola Marymount University; K.A. Maruya, Southern California Coastal Water Research Project. Low-density polyethylene (PE) used for in-situ sampling of organic chemicals in water requires pre-calibration and knowledge of the degree of attainment of equilibrium between the sorbing and aqueous phases. PE-water partition coefficients ( $K_{PEW}$ ) for brominated diphenyl ether (BDE) 47, tetrabromophthalate (TBPH), the DDT metabolites p,p'-DDMU and p,p'-DDNU, methoprene, and PCB congeners 8, 101 and 209 were determined in spiked seawater laboratory experiments. Measured  $K_{PEW}$  values for p,p'-DDMU, PCB 101, and BDE 47 were similar to previously reported values. Log  $K_{PEW}$  was correlated with log  $K_{OW}$  for targeted analytes (log  $K_{OW} < 8$ ). Aqueous concentrations of these chemicals were estimated after correction for non-equilibrium conditions using pre-calibrated PE samplers (containing performance reference compounds) that were co-deployed with caged mussels near large wastewater treatment plant outfalls in Santa Monica Bay and Los Angeles Harbor. PE estimated aqueous concentrations of target analytes in the ng/L range were correlated with mussel soft tissue concentrations, suggesting that PE samplers represent a viable sampling alternative to bivalve sentinels for water quality monitoring of hydrophobic organic compounds.

**471 Occurrence of contaminants of emerging concern in coastal California waters using passive sampling devices** D.A. Alvarez, USGS Columbia Environmental Research Center; K. Maruya, N. Dodder, Southern California Coastal Water Research Project; E. Furlong, USGS NWQL; K. Smalling, USGS; W. Lao, Southern California Coastal Water Research Project. Marine life is constantly exposed to a myriad of organic contaminants originating from urban and agricultural centers along the coast. These chemicals may include legacy organics and contaminants of emerging concern (CECs) which can enter the ocean via surface runoff, rivers and streams, and direct discharge of wastes. Many of the CECs do not accumulate in the tissues of common monitoring species (i.e., bivalves and fish) but may be present at levels in the water to be potentially harmful to sensitive species. Passive sampling devices were deployed at established NOAA Mussel Watch sites along the California coast to provide data on the presence and concentrations of these contaminants. The polar organic chemical integrative sampler (POCIS) was used to sample CECs with a wide range of water solubilities such as pharmaceuticals, fragrances, flame retardants, and current-use pesticides. Solid-phase microextraction (SPME) and polyethylene devices (PEDs) were used to sample hydrophobic contaminants such as PAHs, PCBs, PBDEs, and chlorinated pesticides. The predominant CECs identified were the phosphate-based alternative flame retardants and fragrances. Chemical residues in the passive samplers were positively correlated to the chemical concentrations in mussel tissue, for most of the PCBs, PBDEs, and pesticides sampled by the SPME and PEDs. Out of 34 common chemicals analyzed in both the POCIS and mussel tissue, there were no common detections indicating a low bioaccumulation potential for many of these chemicals. This study indicates that passive sampling techniques can be used as a surrogate for determining the potential exposure of aquatic organisms to a variety of organic contaminants and multiple types of samplers are often needed to be used in concert in order to achieve a holistic assessment of exposure.

**472 The occurrence and fate of chemicals of emerging concern (CECs) in effluent dominated river systems in southern California** A. Sengupta, Southern California Coastal Water Research Project / Biogeochemistry; M. Lyon, D. Smith, Los Angeles Regional Water Quality Control Board; A. Heil, Los Angeles County Sanitation Districts; D. Heil, J. Drewes, Colorado School of Mines; S. Snyder, University of Arizona; K.A. Maruya, Southern California Coastal Water Research Project, SCCWRP. Chemicals of emerging concern (CECs) are a diverse group of relatively unmonitored and unregulated chemicals that are known to occur at trace levels in treated wastewater, yet little is known about their occurrence and fate once discharged into ambient receiving waters. To investigate the concentration and fate of CECs in two effluent-dominated coastal rivers, receiving water samples were collected at sites downstream of municipal wastewater treatment plant (WWTP) discharge points during two dry-weather sampling

events. A reference sample in each watershed was also collected upstream of known WWTP discharge locations to serve as background input of CECs (e.g. via dry weather runoff and atmospheric contributions). All samples were extracted and analyzed for more than 50 pharmaceuticals and personal care products, current use pesticides and flame retarding chemicals using research grade LC-tandem MS and low and high resolution GC-MS. For most CECs, aqueous concentrations in the stream were higher compared to the reference sites, and also decreased with increasing distance from WWTP discharge locations. With the field-collected data, we used a one-dimensional transport – equilibrium chemistry model to parse out the chemical (e.g. photolysis, sorption) and physical (e.g. transport, dilution, water-sediment partitioning) processes that the CECs undergo in these systems.

#### 473 Determination of pharmaceuticals in nearshore marine sediment samples from the Southern California Bight

E.T. Furlong, S.L. Werner, U.S. Geological Survey / National Water Quality Laboratory; A.S. Pait, NOAA/NOS/NCCOS / Center for Coastal Monitoring and Assessment; M. Choi, National Fisheries Research and Development Institute, Korea / Marine Environment Research Division, National Fisheries Development and Research Institute / Research Scientist; K.A. Maruya, Southern California Coastal Water Research Project, SCCWRP; H. Choi, National Fisheries Development and Research Institute. Many coastal urban marine ecosystems receive liquid and solid discharge from wastewater treatment, which was not designed to efficiently remove trace concentrations of the pharmaceuticals and other contaminants of emerging concern that are typically present in discharge. Coastal marine sediments are a likely sink for pharmaceuticals that adsorb to solids by hydrophobic or other interactions, and are a potential means of exposing benthic organisms to these compounds. Consequently, the presence and concentrations of pharmaceuticals were the subject of study at five sites adjacent to wastewater discharges and at 20 sites more widely distributed within the Southern California Bight (SCB), a biologically diverse and productive region that is comprised of the 700 km of recessed coastline from Point Conception, in Santa Barbara County, California to Cabo Colnett, just south of Ensenada, Mexico. Grab samples of surficial sediment were collected, chilled immediately after collection and then frozen prior to analysis. Thawed wet sediment aliquots were extracted by using pressurized liquid extraction with a water/acetonitrile mixture. Following extraction, an aliquot of the sample extract was solvent exchanged into water modified with ammonium formate, filtered, and analyzed for 85 pharmaceuticals by HPLC/MS/MS, using positive electrospray ionization and selected reaction monitoring. Twenty-three pharmaceuticals were each detected in 3 or more samples, with median concentrations ranging between 0.12 and 7.6 nanograms per gram (ng/g). Thirteen pharmaceuticals were detected in one or two samples, and 49 pharmaceuticals were not detected at all. Diphenhydramine, an over-the-counter antihistamine, was detected at the highest concentration, 130 ng/g. The four most frequently detected pharmaceuticals, present in fourteen or more samples, were azithromycin, caffeine, verapamil, and thiabendazole, detected at frequencies of 80, 68, 68, and 56 percent, respectively, with maximum concentrations of 19, 14, 4.5, and 1.7 ng/g, respectively. Overall, pharmaceuticals were detected at higher concentrations and at greater frequency in the five sediment samples collected adjacent to discharges than in the 20 samples collected more widely within the SCB. However, a quantitative relationship between proximity to discharge points and the composition or concentrations of pharmaceuticals detected in sediments could not be discerned.

#### 474 Use of Bioassays to Identify Contaminants of Emerging Concern in Aquatic Ecosystems

D. Schlenk, University of California-Riverside / Department of Environmental Sciences; R. Lavado, University of California-Riverside / Department of Environmental Sciences; J.E. Loyo-Rosales, University of California Berkeley / Department of Civil and Environmental Engineering; W. Jones, University of California Riverside / Department of Environmental Sciences; L.A. Maryoung, University of California Riverside / Department of Environmental Sciences; N. Riar, University of California-Riverside / Department of Environmental Sciences; I. Werner, Swiss Centre for Applied Ecotoxicology, Eawag/EPFL / Department of Anatomy, Physiology and Cell Biology; D.L. Sedlak, University of California Berkeley / Department of Civil and Environmental Engineering. Contaminants of Emerging Concern (CECs) represent a “universe” of known and unknown compounds including pharmaceutical and personal care products, nanomaterials, and human metabolites. Aquatic organisms tend to have the greatest exposure to human-derived CECs due to point source discharge

of domestic wastewater effluents and to non-point source runoff to surface water. Significant uncertainty exists in determining the ecotoxicological risk of these compounds. Some studies have shown that exposure to relatively low concentrations of synthetic hormones have abolished populations of fish. Populations of threatened and endangered species of fish in the San Francisco Bay Delta have shown significant declines leading to changes in water allocations to the Central Valley and Southern California. Using an easily measured endocrine response (estrogenic activity), evaluations of surface waters that drain into the estuary were carried out. Site-specific patterns of activity were observed with causal compounds varying between the Napa River and the Delta of the Sacramento River. Further analyses of the Sacramento River Delta have indicated the potential for pyrethroid insecticides and surfactant mixtures as contributing factors to this response. Whether estrogenic activity is causally linked to population decline is uncertain. However, the temporal association between urban pyrethroid use and population declines in Delta fish species indicates further research is necessary.

#### 475 From ‘omics to otoliths: using *Menidia* species to assess endocrine disruption

S.M. Brander, Bodega Marine Lab, University of California, Davis / Environmental Toxicology, / Department of Biology & Marine Biology; R.E. Connon, University of California – Davis / School of Veterinary Medicine, Aquatic Toxicology Laboratory, University of California, Davis / Anatomy, Physiology and Cell biology; G. He, University of California, Davis / Dept. Environmental Toxicology; J.A. Hobbs, University of California, Davis / Dept. Wildlife, Conservation, Fisheries; K.L. Smalling, U.S. Geological Survey; J.W. White, University of North Carolina, Wilmington / Dept. Biology and Marine Biology; S. Teh, University of California, Davis / School of Veterinary Medicine, Dept. Anatomy, Physiology and Cell Biology; I. Werner, Swiss Centre for Applied Ecotoxicology, Eawag/EPFL / Department of Anatomy, Physiology and Cell Biology; M.S. Denison, University of California, Davis / Dept. Environmental Toxicology; G.N. Cherr, University of California, Davis / Bodega Marine Lab, Departments of Environmental Toxicology and Nutrition. Endocrine disrupting chemicals (EDCs) are known to interfere with endogenous hormones and cause physiological abnormalities in fishes. Both theory and empirical data confirm that EDCs can also cause declines in fish populations. However, few studies have attempted to link complex environmental EDC mixtures with responses at multiple tiers of the biological hierarchy, including population-level effects. Additionally, the use of environmentally relevant fishes as surrogate species from regions with demonstrated fish population decline, such as the San Francisco Bay and Sacramento San Joaquin Delta, is lacking. This is of particular importance to endangered or threatened fish species that cannot be sampled in sufficient numbers from the wild to determine whether endocrine disruption is playing a role in their decline. To this end, we undertook a four-tiered investigation into estrogenic and androgenic EDC effects in *Menidia audens* (Mississippi silverside), an Atherinid distributed throughout the impacted San Francisco Bay (SFB) estuary. Correlations were observed between biological scales that indicated *Menidia* are impacted by the complex EDC mixtures present in Suisun Marsh, SFB at the molecular, organism and population levels. Results from laboratory exposures to pyrethroid pesticides commonly detected in the SFB using *Menidia beryllina* (inland silverside), which indicate that the pyrethroids bifenthrin and permethrin can act as estrogens or anti-estrogens, lend support to field observations. We used the data collected from these and other ongoing research efforts to parameterize a population dynamic model. This model includes the functional relationship between EDC effects, sex ratio, and mating success and provides a general, analytical understanding of the potential effects of EDCs on population persistence in *Menidia* and other impacted group-spawning species, such as the Delta smelt.

#### 476 The Role of Contaminants, in the Context of Multiple Stressors, on the Collapse of the Striped Bass Population in the San Francisco Estuary

D.J. Ostrach, University of California at Davis / Pathobiology, Conservation & Population Biology Laboratory, Ostrach Consulting / Pathobiology, University of California at Davis / Center For Watershed Sciences. This investigation into the collapse of the striped bass population in the San Francisco Estuary (SFE) began in the late 1980s by addressing the starvation hypothesis. Our findings ruled out the starvation hypothesis but uncovered significant sublethal contaminant effects. It was thought that the decline was due to hydrodynamic changes relating to Delta outflows, effects of the State and Federal water projects and an invasive clam potentially limiting food supply for pelagic larvae. However, the combined effects of these



factors did not explain the collapse of striped bass or other pelagic fish in the SFE. Most agency research efforts continued to focus on uncovering a major cause of the collapse to determine management action. As investigations continued over the next 15 years, management and researchers began to accept that multiple stressors and associated interactions contributing to the decline of pelagic fish in the SFE ecosystem were complicated, multifaceted and research efforts were redirected accordingly. In 2005 as part of the Interagency Ecological Program Pelagic Organism Decline investigations, I assembled a research team with expertise in histopathology, analytical chemistry, geochemistry, toxicology, protein profiling, ecological modeling, spatial mapping, microbiology and parasitology. The goal of this research program was to assess the significance of contaminants relative to other stressors on the decline of the striped bass population. Significant progress was made over a three year investigation and identified contaminants as an important stressor on striped bass throughout the first 6-8 months of life. Maternal transfer of xenobiotics and adverse effects was documented over an extended period of time likely causing population level effects. Findings of abnormal disease and parasitism were found in juvenile striped bass in all three years studied and are considered to have a significant impact on the health status of the fish and subsequently the population. In addition, data indicated adult striped bass are also adversely affected by the bioaccumulation of contaminants such as PBDEs and heavy metals. This interdisciplinary multiple stressor approach yielded significant findings on the role of contaminants in the decline of striped bass in the SFE in a relatively short period of time. This study illustrates the utility and importance of using this type of approach to investigate contaminant effects in complex ecosystems.

**477 Habitat Use of Striped Bass in the San Francisco Estuary, and Their Relation to Total Mercury and Heavy Metal Body Burden Upon Capture** J.H. Walsh, Moss Landing Marine Laboratories; J. Hunt, J. Davis, San Francisco Estuary Institute; G.M. Cailliet, Moss Landing Marine Laboratories; D. Ostrach, Ostrach Consulting. Striped bass (*Morone saxatilis*) is a resident anadromous fish in the San Francisco Estuary (SFE) encountering anthropogenic contaminants due to habitat proximity to pollution sources. Habitat use has been shown to be an important factor in the bioaccumulation of contaminants in striped bass. Several studies have shown that contaminants are likely in part responsible for the decline of this striped bass population and pose potential health risks to consumers. In this study we performed an integrated assessment of concentrations of metals in striped bass fillets, and used strontium isotope ratios from otoliths to distinguish freshwater, estuary, and marine habitat use. This approach, using recently developed methods integrate habitat use and contaminant data, provides a new and more accurate approach to diagnosing and analyzing contaminant effects in striped bass. Results indicate that mercury and selenium are at or approaching levels of concern in striped bass tissue, and mercury tissue concentration increased over time. Findings also indicated that cadmium levels in fillets, normally bioaccumulated in visceral organs, were detected in a third of the samples. Furthermore, we document further evidence of a freshwater resident subpopulation and determined that distinct subpopulations exist based on habitat use. When results from these methods are integrated, there was also indirect evidence that striped bass females are passing significant amounts of mercury into their eggs. The data indicate that several contaminant stressors continue to pose a serious threat to recovery of striped bass in the SFE. Habitat use of individual striped bass also needs to be addressed in future surveys estimating population size and to identify if certain striped bass subpopulations are more at risk from contaminants. The results and approach used in this study will aid ecosystem managers in better understanding and addressing contaminant effects in San Francisco Estuary pelagic fish species.

**478 Transcription Profiling in Delta Smelt (*Hypomesus transpacificus*): Up- and Downstream Exposures from a Wastewater Effluent** R.E. Connon, University of California – Davis / School of Veterinary Medicine, Aquatic Toxicology Laboratory, University of California, Davis / Anatomy, Physiology and Cell biology; M. Hasenbein, Technische Universität München / Aquatic Systems Biology Unit, Department of Ecology and Ecosystem Management; I. Werner, Swiss Centre for Applied Ecotoxicology, Eawag/EPFL; E. Fritsch, University of California, Davis / Molecular Biosciences; A. Javidmehr, L. Deanovic, University of California, Davis / Anatomy, Physiology and Cell biology; S. Beggel, Technische Universität München / Aquatic Systems Biology Unit, Department of Ecology and Ecosystem Management; E.R. Hudson-Davies, University of California, Davis / Anatomy, Physiology and Cell biology; J. Geist, Technische Universität

München / Aquatic Systems Biology Unit, Department of Ecology and Ecosystem Management. The delta smelt (*Hypomesus transpacificus*) is an endangered fish species endemic to the Sacramento-San Joaquin Estuary, California; populations of which have significantly declined over the past three decades. Contaminant exposure is one of many likely contributors to the decline. We investigate transcriptional responses in larval delta smelt resulting from exposure to water samples collected at the Department of Water Resources Field Station at Hood, a site of concern situated upstream of known delta smelt spawning habitats, and downstream of the Sacramento Regional Wastewater Treatment Plant (SRWTP). Microarray assessments indicate impacts on the energy metabolism, DNA and RNA processing, the immune system, development and upon activity. Transcription responses of fish exposed to water samples from Hood were compared with exposures to 9% effluent samples from SRWTP, water from the Sacramento River at Garcia Bend (SRGB); upstream of the effluent discharge, and SRGB water spiked with 2 mg/L total ammonium; 9% effluent equivalent. Results indicate that transcriptomic profiles from Hood are similar to SRWTP effluent and ammonium spiked SRGB water, but significantly different from SRGB, however, SRGB samples were also significantly different to laboratory controls, suggesting that SRWTP effluent is not solely responsible for the responses determined at Hood, that ammonium exposure likely enhances the effect of multiple-contaminant exposures, and the observed mortality at Hood is due to the combination of effluent discharge including contaminants arising from upstream of the tested sites. This study shows that transcriptomic responses of fishes can be valuable endpoints for identification of sources of toxic compounds in surface waters that occur at sublethal concentrations.

**479 The effects of a commonly used pyrethroid pesticide, bifenthrin, on the reproduction of steelhead (*Oncorhynchus mykiss*)** K.L. Forsgren, University of California, Riverside / Department of Environmental Sciences, University of California / Dept of Environmental Sciences; N. Riar, D. Schlenk, University of California-Riverside / Department of Environmental Sciences. The San Francisco Bay Estuary and Sacramento-San Joaquin Delta (Bay-Delta) is an important breeding and nursery ground for many species. Habitat quality and contamination of surface waters are limiting factors for ESA-listed fish stocks in watersheds with significant land use. Bifenthrin has received little attention regarding its effects on salmonid populations despite being detected in northern California runoff. While the potential for aquatic toxicity is evident, it is unknown what effect bifenthrin exposure has on the reproductive health of fishes. Plasma sex steroids and gonadosomatic index (GSI) were determined in juvenile steelhead exposed to bifenthrin (low:  $0.028 \pm 0.006$  ug/L; high:  $0.719 \pm 0.073$  ug/L) for 14 days and gonadal tissue was examined histologically. In male steelhead, sex steroids were not significantly altered (E2  $p = 0.0634$ , T  $p = 0.0833$ , 11-KT  $p = 0.3057$ ) after treatment. GSI was reduced ( $p = 0.0231$ ), but the testis did not show measurable histological damage. Female steelhead exposed to bifenthrin (high dose) had significantly ( $p = 0.0251$ ) elevated estradiol-17b (E2) levels. There was no difference in testosterone (T;  $p = 0.1430$ ), 11-ketotestosterone (11-KT;  $p = 0.0760$ ) or GSI ( $p = 0.1937$ ). Although ovarian follicle diameter significantly ( $p < 0.0001$ ) increased in bifenthrin-treated fish, widespread atresia was observed throughout the ovary (low dose  $91.24 \pm 8.89\%$ ; high dose  $82.76 \pm 10.84\%$ ). These data indicate that exposure to bifenthrin results in reproductive dysfunction in female steelhead while males appear to be relatively unaffected. The ecological implications are uncertain, but further study is warranted. Additionally, given the increased urban use of bifenthrin and relatively high levels in the aquatic environment, a more comprehensive understanding of the impact of bifenthrin on wildlife will be imperative for improving risk assessment of pyrethroid use in the Bay-Delta in coming years.

**480 Current-Use Pesticides in the San Francisco Bay-Delta Estuary: Exposure to Complex Mixtures and Multiple Environmental Compartment** M.L. Hladik, K.L. Smalling, J.L. Orlando, K.M. Kuivila, U.S. Geological Survey. Current-use pesticides pose a potential threat to aquatic organisms in the San Francisco Bay-Delta Estuary, an ecologically important habitat and a location for large-scale ecosystem restoration. It is critical to understand the effects of pesticides on this ecosystem, which requires a detailed knowledge of pesticide exposure throughout the watershed. Pesticides are transported into the estuary dissolved in the water or bound to the sediments. Recent studies have shown that aquatic organisms of concern can accumulate current-use pesticides; these organisms are exposed to pesticides

via water, sediments, and food. Hundreds of pesticides are applied annually within the San Francisco Estuary watershed on a variety of crops and in urban areas. As a result of these diverse inputs, aquatic organisms are exposed to complex mixtures of pesticides that vary spatially and temporally. Pesticide use changes over time as older pesticides are withdrawn, new pesticides or new uses for pesticides are registered, and new pests become a problem. Monitoring programs need to adapt to these changing use patterns to be effective. The U.S. Geological Survey's analytical methods are routinely updated and currently include over 100 pesticides and pesticide degradates in all relevant environmental compartments (water, sediment and tissue) using GC/MS and LC/MS/MS. Case studies from our current and past research efforts will be highlighted. Pyrethroid insecticides are usually bound to sediments and their high aquatic toxicity requires low detection limits; therefore, special methods were developed for these compounds. The number of fungicides used is increasing with the registration of new active ingredients, and 34 are included in our current methods. Rice, an important crop in this watershed, has seen a change in herbicide applications after molinate was phased out in 2009; this is reflected by increased detections of propanil (and its degradate) and clomazone. Increasing both our knowledge of pesticide occurrence and our collaborations with toxicologists, ecologists, and fisheries biologists will aid in linking pesticide exposure to effects on aquatic organisms within multi-stress environments such as San Francisco Bay-Delta Estuary.

**481 A comprehensive study of the occurrence of pyrethroids in water and sediment from the lower American River** S. Clark, S. Ogle, Pacific EcoRisk; T. Albertson, Caltest Analytical Laboratories; C. Harbourt, G. Hancock, Waterborne Environmental; G. Mitchell, FMC Corporation; A. Barefoot, D. Tessier, DuPont Crop Protection; M.G. Dobbs, Bayer Crop Science, Bayer CropScience / Ecotoxicology Group; P. Hendley, Syngenta Crop Protection, LLC. / Senior Syngenta Fellow, Syngenta Crop Protection, LLC. / Product Safety; K.S. Henry, Syngenta Crop Protection, Inc. / Ecological Sciences. The American River is a high quality water source used for the Sacramento metropolitan area. In a previous study in which grab samples of water were collected from the river bank during 4 rain events and one dry weather event, pyrethroid insecticides were detected in water samples collected over a 30-km reach of the lower American River at concentrations that were reported to be toxic to the amphipod *Hyaella azteca*. In October 2011, a follow-up monitoring study was initiated by the Pyrethroid Working Group with the goal of providing a more robust assessment of pyrethroid concentrations in water and sediment of the lower American River. Water samples were collected during 3 rain events and 2 dry events along cross-river transects at 7 locations, with 5 stations per location and 3 depths per station, resulting in a maximum of 105 samples per event. Sediment samples were collected at each station during a dry weather event. All water and sediment samples were analyzed for bifenthrin, cyfluthrin, lambda-cyhalothrin, cypermethrin, deltamethrin, esfenvalerate, fenpropathrin, and permethrin. All pyrethroids were less than the reporting limit (

**482 Pyrethroid concentrations in the American River: historical assessment and impact of proposed regulatory controls** T.M. Young, University of California-Davis / Dept. of Civil & Environ. Engineering; B. Jorgenson, University of California, Davis / Agricultural and Environmental Chemistry. A watershed pyrethroid insecticide exposure model was developed for the lower American River watershed located in California, USA. The model incorporated empirically derived washoff functions based on previously run small scale rainfall simulations, along with actual pyrethroid insecticide use and watershed properties for Sacramento County, California. The model was calibrated to in-stream monitoring data and utilized to predict daily river pyrethroid concentration for a period spanning 1995 through 2010. Based on model predictions, a marked increase in pyrethroid toxic units is observed starting in the calendar year 2000, coincident with a watershed-wide increase in pyrethroid use. Approximately 80% of the predicted toxic unit exposure in the watershed was associated with the pyrethroids bifenthrin, cyfluthrin, and cypermethrin. Pyrethroid applications for above-ground structural pest control purposes utilizing suspension concentrate categorized product formulations accounted for greater than 93% of the total toxic unit exposure for all modeled years except 1995. Application of mitigation strategies, such as curtailment of structural perimeter band and barrier treatments as proposed by the California Department of Pesticide Regulation, yielded an approximate 80% reduction in predicted total toxic unit exposure in all modeled years. The model also predicted that similar

mitigation gains could be achieved through a switch from suspension concentrate categorized products to emulsifiable concentrate categorized products. Even with these mitigation gains, the predicted concentration of some pyrethroids would continue to exceed chronic aquatic life criteria for pyrethroids on a frequent basis, illustrating the recalcitrant nature of the pyrethroid problem.

**483 Predicting the effectiveness of label changes in reducing pyrethroid loads to the American River** W.W. Williams, G. Hoogeweg, Waterborne Environmental, Inc; D. Denton, U.S. EPA Region 9 / c/o SWRCB; P.L. TenBrook, U.S. Environmental Protection Agency, Region IX; R. Breuer, California Department of Water Resources / Environmental Water Quality and Estuarine Studies; K.D. Moran, TDC Environmental, LLC. Attention has been drawn to pyrethroid detections in the American River and other waterways draining urban land uses in California. In response, the California Department of Pesticide Regulation has required changes in pyrethroid labels limiting structural uses to spot and crack treatments. The potential washoff of pyrethroid residues were estimated for urban areas draining to the American River from 10 years of historical pesticide use records under pre- and post- label changes. The results indicate that significant reductions in pyrethroid loads to the American River are likely to occur under the modified application practices.

**484 Assessing Stormwater Impacts on Coastal Preserves: An Integrated Approach in the La Jolla Area of Special Biological Significance, San Diego, CA** S. Gruber, Weston Solutions, Inc., Weston Solutions; D. McCoy, Weston Solutions, Inc.; C. Stransky, AMEC Earth and Environmental, AMEC Earth & Environmental / Aquatic Sciences, AMEC Environment & Infrastructure / Senior Aquatic Scientist; R. Schottle, AMEC Environment & Infrastructure; S. Jarrell, Scripps Institution of Oceanography. The variable nature of stormwater runoff presents unique challenges with regard to accurately characterizing water quality and potential receiving water impacts. A multiple lines of evidence (MLE) approach is needed to accurately characterize potential biological impacts and stressors of concern to cost-effectively identify future Best Management Practices (BMPs). This is particularly true when assessing stormwater impacts on coastal marine preserves such as Areas of Special Biological Significance (ASBS), where reliance on water quality monitoring alone may be unprotective of the unique beneficial uses found in these areas. State regulators in California have recently adopted Special Protections for these areas that rely on a MLE approach for assessment. Results of an integrated, MLE approach currently being conducted in two adjacent ASBS (ASBS 29 and 31, collectively referred to as the La Jolla ASBS) will be showcased in this presentation. The goal of the program is to assess the potential impacts of stormwater runoff on different coastal habitats to better understand the extent to which the ASBS Special Protections are truly protective of beneficial uses. The assessment used an integrated approach, which focused on four lines of evidence: 1. water chemistry (stormwater and receiving water constituents monitored over the course of a storm); 2. toxicity to native marine species (*in situ* toxicity, laboratory toxicity, and Toxicity Investigation Evaluations (TIEs)); 3. bioaccumulation with several native marine species; and 4. biological community measures of potential impairment associated with stormwater impacts. Initial results of the multi-year study have shown that potential impacts to the ASBS from stormwater is highly dependent upon the inter-related dynamics of tidal stage, receiving water habitat, proximity to storm drain outfalls, and cross-contamination from discharges well outside of the ASBS. These site specific variables are important considerations when assessing potential stormwater impacts on the ASBS and need to be considered when implementing and interpreting results of more general monitoring required by the Special Protections. The results from this study have important implications for managing stormwater discharges to ASBS with respect to new the new regulations designed to protect the beneficial uses of these important coastal resources.

**485 Comparison of Modeled Biological Condition of California Streams at Statewide and Regional Geographic Scales** J.T. May, US Geological Survey / California Water Science Center; L.R. Brown, US Geological Survey; R.D. Mazor, Southern California Coastal Water Research Project; A.C. Rehn, P.R. Ode, California Department of Fish and Game; I.R. Waite, US Geological Survey. The ability to predict changes in biological condition of California streams in response to stressors such as climatic variation and human development is becoming increasingly essential for establishing resource management objectives and strategies. We used boosted regression

trees (BRT) to model stream biological condition, as measured by predicted benthic macroinvertebrate taxonomic completeness, (the ratio of observed to expected, O/E models). BRT models are robust, requiring few assumptions compared with traditional modeling techniques such as multiple linear regression. The BRT models were constructed to provide baseline support for the development of numerical biological objectives for California's Wadeable Streams by identifying important environmental and stressor variables statewide and for eight regions within the state. Regions were defined on the basis of ecological, hydrologic, and political factors and roughly corresponded with ecoregions. Environmental and stressor variables were derived from geographic information system coverages. The statewide model explained 55% of the variance and identified the composite measure of anthropogenic disturbance (the sum of urban, agricultural, and managed vegetation land cover) as the most important variable. The individual regional models explained from 57 to 87% of the variance. Measures of human disturbance were important in the three coastal regions. Along the south and central coast, near-site measures of urbanization were most important. In the north coast region, the composite measure of disturbance at the watershed scale was most important. In the two mountainous regions, natural gradients were most important, including slope, precipitation, and temperature. The remaining three regions had relatively small sample sizes ( $n \leq 75$  sites) giving mixed results. The statewide model could be used to predict biological conditions at unsampled sites using a consistent set of predictor variables, which may be useful for planning and management purposes. The regional models provide a more specific understanding of regionally important environmental and stressor gradients.

**486 Developing sediment contaminant objectives protective of all beneficial uses: Examples from San Diego Bay, CA** J. Diamond, Tetra Tech, Inc.; M. Bowersox, Tetra Tech, Inc. / Laboratory Department; J. Flippin, Tetra Tech, Inc. / Center for Ecological Sciences; C. Boschen, Tetra Tech, Inc.. Biological and chemical sediment data indicated impairments in areas of San Diego Bay, CA, prompting the need for a total maximum daily load (TMDL) for certain contaminants including PAHs, PCBs, and chlordane. These contaminants have the potential to bioaccumulate and thereby have detrimental effects on wildlife and humans. San Diego Bay provides many beneficial uses including aquatic life propagation, wildlife habitat and propagation, and fish and shellfish harvesting for human consumption (both recreational and subsistence). While a variety of sediment quality objectives (SQOs) are available for the contaminants of interest based on aquatic life protection (including some that are region-specific), it was not clear whether these SQOs are protective of wildlife and humans. Also, these contaminants lack or have uncertain sediment screening levels for indirect exposures to wildlife and humans. To address these challenges, an ecological and human health risk assessment process was used to identify appropriate sediment targets for wildlife and humans, respectively. For human exposure, a set of sediment targets were identified for each contaminant based on different risk factors. Depending on the exposure assumptions, the cancer risk level, and the biota-sediment accumulation factor (BSAF) used, SQOs based on aquatic life may or may not be protective of human consumption of fish and shellfish in the bay. Certain SQOs based on aquatic life toxicity were protective of all uses for chlordane but for PCBs and PAHs, ecologically based SQOs could be an order of magnitude higher than targets based on cancer risk. Having accurate, defensible data regarding BSAFs and exposure is critical to developing appropriate sediment targets for these contaminants in waterbodies having significant fish and shellfish consumption uses.

**487 Effects of Southern California Wildfires on Storm Water Contaminant Runoff** E.D. Stein, Southern California Coastal Water Research Project / Biology department; J. Brown, Southern California Coastal Water Research Project; T. Hogue, M. Burke, University of California, Los Angeles. Periodic wildfires are a natural component of southern California's forest and scrubland and are essential to maintaining overall ecological health of these systems. However, relatively little attention has been paid to the effects of post-fire runoff on downstream loading of pollutants, such as metals and organic compounds. These compounds may be of particular interest if burned areas drain to impaired waterbodies. In addition to the direct effects of runoff from burned landscapes, the materials left behind in ash at the burn location can be carried away from the fire in smoke and ash. Subsequent atmospheric deposition can markedly increase the quantity of various constituents available to storm flows downwind of fires. The goal of this study is to assess regional patterns of runoff and contaminant loading

from wildfires in urban fringe areas of southern California. Post-fire stormwater runoff was sampled from five wildfires that each burned between 115 and 658 km<sup>2</sup> of natural open space between 2003 and 2009. Between two and five storm events were sampled per site over the first one to two years following the fires for basic constituents, metals, nutrients, TSS, PAHs and various halogens. Results were compared to data from 16 unburned natural areas and 6 developed sites. Mean copper, lead and zinc flux (kg/km<sup>2</sup>) were between 112 and 736 fold higher from burned catchments and total phosphorous was up to 921 fold higher compared to unburned natural areas. PAH flux was four times greater from burned areas than from adjacent urban areas. Ash fallout on nearby unburned watersheds also resulted in a three-fold increase in metals and PAHs. Attenuation appears to be driven mainly by rainfall magnitude.

**488 Incorporating nonperennial and ephemeral streams in bioassessment programs** R.D. Mazor, C. Solek, Southern California Coastal Water Research Project; E.D. Stein, Southern California Coastal Water Research Project / Biology department; P. Ode, CA Department of Fish and Game / OSPR; A.C. Rehn, California Department of Fish and Game. Nonperennial streams comprise the majority of stream length in coastal southern California, but most stream assessment tools were developed for perennial streams. Consequently, many bioassessment programs in California exclude nonperennial streams from their purview, and leave much of the region unassessed. To address this gap, we conducted a two-part pilot study in the San Diego region. First, we compared extent estimates the National Hydrography Dataset Plus (NHD Plus) with those derived from ambient surveys to see if maps could correctly identify the extent and location of nonperennial streams. Surveys found that nonperennial streams were less extensive than estimated by the NHD Plus, but still comprise large portions of the region (73% vs. 90% for NHD Plus). The discrepancies may be caused by data limitations within the NHD Plus, as well as by changing conditions, as site visits suggest that nonperennial streams in urban areas have been converted to perennial flows. Second, the validity of the Southern California Index of Biotic Integrity (IBI) was evaluated by repeated sampling at 12 nonperennial and 3 perennial sites representing a gradient of stress. Flow status does not preclude high IBI scores, and low stress nonperennial sites had scores similar to those observed at low stress perennial sites. Furthermore, the IBI declined as stress increased. The IBI was stable at most sites both within and between years, but sharp declines were observed at high stress sites. These declines were associated with declines in discharge, fast water habitat, and (to a lesser extent) increases in sands and fines and macroalgae cover. These findings suggest that the IBI can accurately assess nonperennial streams represented in the study, and that it responds to stress in a predicted manner. These findings support the use of the IBI to assess nonperennial streams, although additional sampling at a large number of reference streams will determine if any refinements to the IBI are necessary. Future research will focus on streams with highly ephemeral flow, where new assessment tools based on other biological indicators will need to be developed.

**489 Regional Assessment of Trash in Southern California Streams** T. Von Bitner, Orange County Public Works. Stormwater agencies have recognized trash as a key stressor to water quality conditions in southern California, but there was no coordinated effort to develop a consistent method of estimating trash loadings, understand its pathways into the environment and to identify and prioritize remediation efforts at the watershed scale. To address this issue, in 2011 the Southern California Stormwater Monitoring Coalition Regional Monitoring Program (SMCRMP) included trash to its list of indicators used to identify key stressors to stream health throughout the region. The SMCRMP stream assessment program is a probability based sampling program designed to assess the ambient condition of the regions 15 major watersheds through a collaborative effort among stormwater agencies who annually sample for benthic macroinvertebrates, attached algae, water chemistry and toxicity, physical habitat conditions. The goal of the Trash Assessment Pilot Project (TAPP) was to improve our understanding of the spatial distribution and magnitude of the trash problem at both the regional and local scales and to examine the sources of trash and its pathways into streams. During the 2011 survey 77 sites were assessed for trash with a total of 2087 pieces of trash counted. Urban streams contained more trash than open space streams and the top three categories of trash included 60% plastic items, 11% glass items, and 7% metal items. The most abundant item counted were plastic bags followed by Styrofoam, and plastic food wrappers. In all, the top five items constituted 60% of all items



collected across southern California streams. These results are being used by state and local agencies to determine the best management approaches for dealing with this wide ranging problem. The 2011 TAPP was made possible by a multi-partner collaboration involving the SMC member agencies, the three southern California Regional Water Quality Control Boards, the State of California Surface Water Ambient Monitoring Program (SWAMP), the Department of Fish and Game, and the Los Angeles and San Gabriel Rivers Council for Watershed Health.

**490 Sources and Effects of Endocrine Active Compounds in Urbanized Aquatic Environments** H. Schoenfuss, St. Cloud State University / Aquatic Toxicology Laboratory, St. Cloud State University / Department of Biological Sciences WSB-273, St. Cloud State University / Department of Biological Sciences MS-273; T. Minarik, Metropolitan Water Reclamation District of Greater Chicago; D. Martinovic, University of St. Thomas; M. Schultz, The College of Wooster. Urban aquatic ecosystems often lack the upstream/downstream dichotomy of undisturbed watersheds. Furthermore, sources of aquatic pollution can be multifold complicating efforts to link sources and biological effects. Over four years we assessed endocrine active compound (EAC) presence and biological effects across a large urban aquatic ecosystem. Specifically, we measured EACs in the effluent and receiving waters, conducted quarterly total estrogenicity assays, and analyzed in excess of 5,000 fish tissues from wild-caught and laboratory reared animals for the presence of the egg-yolk protein vitellogenin (vtg) and for histopathological changes. Steroidal estrogens (E1, E2, E3) were frequently detected at very low ng/L concentrations across sampling sites while nonylphenol (NP) was more variable and often found at high ng/L concentrations especially in treated wastewater effluent. Our study documented estrogenic activity throughout the system (0.4-7.8ng/L estradiol equivalency), that wastewater treatment plant effluent contributes significant EAC loads to the aquatic ecosystem, and that these contributions are less temporally variable than non-point sources of EACs. Male sunfish caged downstream of major water reclamation plant effluents exhibited a slight, but significant increase in plasma vtg concentrations, however, the presence of vtg in upstream caged fish suggests additional sources of EACs. Plasma vtg in male sunfish caged for 14 days was usually 25-50% lower than in wild caught sunfish at the same site, indicating that no exposure plateau had been reached. Interestingly, while exposed male fish responded with the to-be-expected induction of the egg-yolk protein vitellogenin (a common biomarker in male fish exposed to estrogenic EACs), they did not develop histopathological changes to their reproductive organs (i.e., intersex) that have been reported in more pristine aquatic ecosystems that are subject to sudden influx of wastewater discharge. The absence of widespread histopathological changes suggesting organismal adaptation in historically effluent dominated aquatic ecosystems. The comprehensive assessment used in this study is well suited to understand how EACs enter the urban environment and may affect resident fish populations. Funding by the Metropolitan Water Reclamation District of Greater Chicago.

**491 Toward an assessment of anthropogenic impacts in two sectors of the Salado Estuary (Ecuador) subjected to different levels of environmental stressors** P. Calle Delgado, Escuela Superior Politecnica del Litoral / Facultad de Ingeniería Marítima y Ciencias del Mar (FIMCM), Escuela Superior Politecnica del Litoral / Department of Marine Engineering Biological, Ocean Sciences and Natural Resources; L. Monserrate, Escuela Superior Politecnica del Litoral / Department of Marine Engineering Biological, Ocean Sciences and Natural Resources; F. Medina, Escuela Superior Politecnica del Litoral; J. Landivar, Escuela Superior Politecnica del Litoral / Department of Marine Engineering Biological, Ocean Sciences and Natural Resources. The Salado Estuary is part of the Gulf of Guayaquil, which is the largest and more productive estuarine system of the South America western coast. The present study, evaluated the physical, chemical and biological conditions of two sectors of this Estuary differing in anthropogenic influences during the dry and wet seasons of 2008 and 2009 respectively. The first sector is the section of the estuary within an industrial-urbanized zone, the city of Guayaquil (SAC), and the second sector is within a protected area, the Reserve of Faunal Production El Salado Mangroves (RPFMS). Measurements of temperature, salinity, dissolved oxygen (DO), pH, hydrogen sulfide, and total dissolved solids (TDS) were measured *in situ*. Samples for ammonia, macrobenthos, and sediment (composition and heavy metals, including lead and mercury) analysis were also collected. Severe hypoxia, anoxia and high fluctuation of salinity occurred in SAC. The two sectors

differed significantly for DO, salinity, TDS, % silts and clays, and species diversity. These differences occurred primarily in the dry season, with only pH and % of silts sediment composition differing in the wet season. Species richness for SAC (8 species) and RPFMS (21 species) was greater in the dry season and declined in the wet season (4 and 11 species, respectively). The most abundant organisms during the dry season for both sectors were benthic annelids. Tubificid oligochaetes were the dominant species making up 98.6% of total macrobenthic community for SAC during the dry season and accounting for 79.4% during the wet season. Individuals from the phylum Nematoda were the most abundant species for RPFMS during the wet season. The Sediment analysis revealed concentrations of lead were significantly higher in SAC. High sediment concentrations of mercury exceeded Ecuadorian sediment thresholds, and the Effects Range Low (ERL) and Effects Range Median (ERM) sediment quality guidelines in both sectors. The degree of contamination and disturbance of benthos is greater in SAC than in RPFMS; however, the RPFMS showed mERMq (Effects Range-Median Quotient) values above 0.144, indicating negative impacts to the benthos. Environmental management strategies and precautionary approaches are required to be enforced by the local authorities to lessen the magnitude and severity of anthropogenic impacts to the Salado Estuary.

**492 Increasing the global relevance of Ni Biotic Ligand Models in aquatic effects assessments of nickel** C.E. Schlekut, E. Rogevich Garman, NiPERA; G. Merrington, WCA Environmental Ltd.; K. De Schampheleere, Laboratory of Environmental Toxicology and Aquatic Ecology, Gent University. Nickel is a priority substance under the European Union's Water Framework Directive (WFD). A bioavailability-based Environmental Quality Standard (EQS) has been proposed for Ni under the WFD. Compliance with the bioavailability-based EQS will be determined by using a user-friendly tool based on Ni Biotic Ligand Models (BLMs). The user-friendly tool, known as Bio-Met ([www.bio-met.net](http://www.bio-met.net)), simplifies the complex speciation, ecotoxicity normalization, and Species Sensitivity Distribution calculations that are necessary when using the full BLMs. The user-friendly version requires a limited number of water quality parameters (pH, Ca<sup>2+</sup>, and dissolved organic carbon (DOC)), thereby allowing for high throughput of samples to facilitate regulatory compliance checking. While the Bio-Met tool is scientifically robust, both the tool and the underlying BLMs have limitations that constrain their application domains. For example, the Ni BLMs have been validated to a pH of 8.2, whereas many European waters have higher pH ranges. A set of comparative toxicity tests was performed on five aquatic species to extend the pH boundary to 8.7. Results indicated that the increase in Ni toxicity with increasing pH was greater than would have been predicted from previous tests at pH ranges below 8.2, illustrating the need to perform such studies. Incorporating these results into Bio-Met will extend the pH boundary and cover most surface waters in Europe. Ni BLMs have only been validated in Europe and in the United States, raising concerns about the relevance of the models to waters and ecological communities in other geographic regions. To this end, the broad geographic validity of the Ni BLMs will be evaluated by determining if the BLMs can predict Ni toxicity to Australian aquatic species in natural Australian waters that exhibit broad ranges of pH, Ca<sup>2+</sup>, and DOC. Given the relative evolutionary isolation of Australian ecological communities, and the unique geochemical characteristics of Australian surface waters, validating the current Ni BLMs to Australian species in Australian waters will be a powerful step in terms of harmonizing bioavailability-based approaches for assessing aquatic risks of Ni.

**493 Integrating algal physiological interactions within the Biotic Ligand Model** M. Lavoie, University of Quebec / INRS-ETE; C. Fortin, INRS – Eau, Terre et Environnement / Université du Québec, University of Quebec / INRS-ETE; P.G. Campbell, University of Quebec / INRS-ETE. The current version of the Biotic Ligand Model (BLM) ignores the effects of essential and non-essential metals on the physiology of organisms and their repercussions on long-term metal uptake and toxicity. This study aimed to evaluate the effects of essential trace metals (Co, Cu, Fe, Mn, Zn), Ca and one non-essential metal (Cd) on the kinetics of membrane-bound transport systems, in order to predict chronic cadmium toxicity in the freshwater alga, *Chlamydomonas reinhardtii*. To do so, short-term Cd and Zn accumulation kinetics were measured for algae acclimated and exposed to different concentrations of free essential metals and to the presence/absence of Cd<sup>2+</sup>. Cadmium accumulation and toxicity was also measured after long-term exposures (60 h). Short-term experiments have shown that Cd

uses three different transport systems (Cd-1, Cd-2 and Cd-3) and that an increase in free  $\text{Zn}^{2+}$  and  $\text{Ca}^{2+}$  concentrations non-competitively inhibited Cd accumulation by approximately 30 to 60%. Moreover, an increase in the concentrations of all five trace metals combined within the acclimation medium led to a significant decrease (by around 3.8-fold) of the Cd binding affinity to the Cd-2 transport systems. We also found that pre-acclimation to  $\text{Cd}^{2+}$  in the presence of low free  $\text{Zn}^{2+}$  and  $\text{Co}^{2+}$  concentrations increased by as much as six-fold the capacities ( $V_{\text{max}}$ ) of the Cd-2 and Zn-2 uptake systems. However, the  $V_{\text{max}}$  of the Cd-2 and Zn-2 transport systems only increased slightly when the cells were acclimated to both high  $[\text{Cd}^{2+}]$  and  $[\text{Zn}^{2+}]$ . These observations are coherent with a Zn nutritional requirement. In the presence of high Zn, transporters are down-regulated due to the relative abundance of Zn leading to a decrease in Cd uptake; in the presence of Cd, transporters are up-regulated to compensate for the relative scarcity of Zn, leading to an increase in Cd uptake. Our biotic ligand model incorporating the physiological effects of Ca, Cd and essential trace metals on Cd transport systems properties predicts steady-state Cd accumulation in the presence of varying concentrations of essential elements reasonably well. Cadmium toxicity can also be accurately predicted as a function of internalized Cd across varying trace metal concentration regimes since algal sensitivity to intracellular Cd was unaffected by the different essential element combinations.

**494 Integration of Biotic Ligand Models (BLM) and Bioaccumulation Kinetics into a Mechanistic Framework for Acute Toxicity Prediction of Metals** K. Veltman, M. Huijbregts, J. Hendriks, Radboud University of Nijmegen / Department of Environmental Sciences. Acute metal toxicity in freshwater organisms is a function of three key factors: geochemical conditions (e.g. DOC), metal-specific properties (e.g. ligand affinity), and species characteristics (e.g. size). The Biotic Ligand Model (BLM) adequately addresses the influence of geochemical conditions and ligand affinity on acute toxicity, but does not yet consider the large influence of species characteristics on toxic response. Also, metal uptake rates are thought to be of key importance in the onset of toxicity and it is a current topic of debate whether the BLM equilibrium framework needs to be refined by a more kinetic approach. In a previous study, we showed that BLM and bioaccumulation kinetics can be integrated into a common, mechanistic framework for metal uptake in aquatic organisms. Metal uptake rate constants were shown to be a function of the metal-specific covalent index, the species-specific ventilation rate and the maximum uptake rate. Metal-specific affinity constants are similar across various aquatic organisms and significantly correlated to the covalent index ( $X^2_{\text{mT}}$ ), which expresses metal affinity for proteins. Furthermore, species-specific ventilation rates scale to body mass to the power  $-1/4$  according to well-established allometric relationships. This implies that if metal uptake rate is an accurate predictor of acute toxicity, than acute toxicity metrics ( $\text{LC}_{50}$ ) should also be quantitatively related to the covalent index and species weight. Here, we provide a quantitative comparison of the mechanistic description for metal uptake with acute toxicity values for organisms belonging to different size classes and with acute toxicity values for different metals. Acute toxicity data ( $\text{LC}_{50}$ ) were empirically collected based on strict selection criteria. The results show that the model accurately predicts acute silver toxicity across organisms belonging to different size classes. Furthermore, it is found that acute toxicity is significantly related to the metal-specific covalent index and that this relationship is similar across various aquatic organisms. However, the relationships, i.e.  $\text{LC}_{50}$  vs  $X^2_{\text{mT}}$ , and uptake rate vs  $X^2_{\text{mT}}$ , are not directly compatible. It is concluded that uptake rate is an important variable in explaining observed differences in toxicity across metals and across species, yet it is not the only explanatory variable. Current efforts focus on model refinement by a more thorough consideration of toxicodynamics.

**495 Mitigation of acute and chronic Ni toxicity to *Hyalella azteca* by Ca and natural organic matter in very soft waters** K. Chan, Wilfrid Laurier U; J. McGeer, Wilfrid Laurier University / Department of Biology. This study is directed on understanding the role of base cations and natural organic matter (NOM) on the recovery of aquatic invertebrates in ecosystems damaged by long term smelter emissions. The goals of the research are to test the validity of biotic ligand principles (competition and complexation) to Ni toxicity in *Hyalella azteca* in very soft waters typical of boreal shield conditions and to understand the role of NOM source on its capacity mitigate against the impacts of Ni. Amphipods (source: Hannah Lake, Sudbury ON) were cultured and tested in soft water (Ca 0.1, Mg 0.05, Na

0.1 and Cl 0.1 (all mM), pH 7.0 and 21°C) following standard methods (Environment Canada EPS1/RM/33). In acute exposures the effects of Ca were tested across a range of concentrations: 0.1, 0.3, 0.75 and 2.0 mM. The  $\text{LC}_{50}$  varied from 0.56 mg/L to 3.2 mg/L demonstrating protective effects of Ca against Ni toxicity. Protective effects were not observed when Mg increased to 0.5 mM and it appeared to act as a stressor as survival of controls was reduced. Dissolved organic carbon concentrations of 6 mg C/L and higher offered significant protection against Ni toxicity. Modelling with the biotic ligand model (BLM) showed that predictions closely matched measured toxicity. Tests with NOM sources collected from boreal waters across Ontario (tested at 6 mg DOC/L) showed that some sources offered more protection than others but there was no clear relationship between NOM optical characteristics (absorbance and excitation emission matrix spectroscopy) and protective capacity. Short term accumulation (6 h) was used to establish linkages between toxicity mitigation and uptake, which followed saturable kinetics. Targeted chronic testing demonstrated that the protective effect of Ca in acute exposures extended to the long term however the degree of protection was proportionally less. In the very soft waters the acute to chronic ratio was approx 50. Chronic effects of Ni were assessed on the basis of both growth (dry weight) and survival. Survival was generally a more sensitive indicator of Ni impacts. Results are considered in the context of the biotic ligand model and understanding the importance of NOM source. This project is supported by the TALER (Terrestrial Aquatic Linkages for Ecosystem Recovery) Research Program, NSERC, Vale and Xstrata.

**496 Modeling uptake of trivalent trace metals by phytoplankton: a case deviating from BLM predictions** A. Cremazy, P.G. Campbell, C. Fortin, INRS-ETE. Major progress has been achieved in recent years in our ability to predict metal bioavailability to aquatic biota. This is due in large measure to the development and improvement of models such as the Biotic Ligand Model (BLM). According to the BLM, provided pH and water hardness are constant, metal bioavailability should be proportional to the free ion activity. The applicability of the BLM to divalent trace metals has been thoroughly investigated since the pioneering work of Sunda and Morel and their co-workers in the 1970s. However, its relevance to trivalent trace metals such as aluminum is still largely unknown. Although the toxic effects of aluminum are relatively well documented, the uptake mechanisms are still speculative and the links between speciation and accumulation remain elusive. This situation is mainly caused by the analytical difficulties encountered when working with trivalent metals, such as the lack of affordable and suitable radio-isotopes and the dearth of sensitive analytical techniques. Scandium (Sc) was used in the present study as a substitute for aluminum, to investigate the applicability of the BLM to trivalent metals. Scandium shares chemical similarities with aluminum and it has a suitable radio-isotope ( $\text{Sc-46}$ , half life of 83.8 days). Sc internalization fluxes in a unicellular alga (*Chlamydomonas reinhardtii*) were determined by kinetic uptake experiments between pH 4.50 and 7.90 and at constant ionic strength. If proton competition is taken into account, Sc internalization fluxes measured between from pH 4.50 to 6.00 are well modeled by the BLM. However, this model underestimates the fluxes at pH  $\geq 6.50$  where Sc speciation is largely dominated by the hydroxo-complexes  $\text{Sc}(\text{OH})_n^{3-n}$  ( $n = 1, 2$  and  $3$ ). Various reasons for this deviation were investigated, including a pH dependency of the potential at the surface of the biological membrane and the contribution of labile Sc hydroxo-complexes through their dissociation in the unstirred layer next to the alga surface. These hypotheses were discarded based on experimental evidence and theoretical considerations. The remaining hypothesis is that the observed deviation from the BLM could be the result of the uptake of one or more undissociated Sc hydroxo-complexes.

**497 Site-specific issues with applying the BLM to evaluate Cu toxicity: overestimation of Cu-DOC complexation and model anomalies in low hardness waters** J.M. Morris, Stratus Consulting; A. Maest, Stratus Consulting Inc.; A. Craven, University of Colorado-Boulder / Chemistry and Biochemistry; J. Lipton, Stratus Consulting Inc.. The Biotic Ligand Model (BLM) was used to estimate concentrations of  $\text{Cu}^{2+}$  in site waters in Alaska and to predict the toxicity of copper to rainbow trout. Visual MINTEQ was also used to predict  $\text{Cu}^{2+}$  concentrations using conditional log K values for  $\text{Cu}^{2+}$ -dissolved organic matter (DOM) binding derived from the same site waters over a range of total copper concentrations. The BLM predicted considerably lower  $\text{Cu}^{2+}$  concentrations than our empirical data when the total copper concentrations were greater than  $1 \mu\text{g L}^{-1}$  ( $2 \times 10^{-8} \text{ M}$ ) under modeled site conditions. The discrepancy could be reconciled by decreasing



the dissolved organic carbon (DOC) input values to the BLM by ~7 times (actual stream value was 2.17 mg C L<sup>-1</sup>). Other researchers have suggested that inputting one-half the measured DOC concentrations to the BLM yields a better fit with fish toxicity data in some cases. These findings suggest that the BLM appears to apply stronger net Cu<sup>2+</sup>-DOM binding across a range of Cu:DOM ratios and water qualities found in many site waters, which could result in an under-prediction of copper toxicity. Additionally, the BLM applies a log K value of 7.4 for the strength of copper binding to the gill (biotic ligand) for rainbow trout. Plots of total copper LC<sub>50</sub> and gill log K values show that a gill log K of 7.4 is close to the inflection point for predicted toxicity, and even small changes in gill log K can produce large changes in predicted copper toxicity. Finally, at hardness values < 20 mg L<sup>-1</sup> as CaCO<sub>3</sub>, the BLM predicts lower Cu toxicity to rainbow trout than at somewhat higher hardness values. The lower predicted toxicity appears to be related to differences in modeled metal binding affinities between the gill and DOC. At very low hardness values (~5 mg L<sup>-1</sup> as CaCO<sub>3</sub>), the BLM predicts that copper will preferentially bind with DOC, and modeled LC<sub>50</sub> values decrease with increasing hardness (e.g., 5-20 mg L<sup>-1</sup> as CaCO<sub>3</sub>). At higher hardness values (e.g., >25 mg L<sup>-1</sup> as CaCO<sub>3</sub>), the LC<sub>50</sub> is predicted to rise by ~4 or 5 mg Cu L<sup>-1</sup> for each ~20-mg L<sup>-1</sup> increase in hardness. There is no empirical evidence to suggest that aquatic biota are more tolerant of metal concentrations at low hardness values, and the hardness-based water quality criteria equations do not produce this peculiarity. A number of headwater streams around the country have low-hardness waters and use of the BLM at those sites should proceed with caution.

#### 498 Use of Multiple Linear Regression to Model the Influence of Water Chemistry on Chronic Iron Toxicity to Freshwater Organisms

B.J. Adams, Rio Tinto / Product Stewardship; P.H. Rodriquez, Centro de Investigación Minera y Metalúrgica (CIMM); A. Cardwell, Oregon State University; L.M. Tear, Windward Environmental LLC; W.A. Stubblefield, Oregon State University; K.V. Brix, McMaster University; D.K. DeForest, Windward Environmental LLC. Chronic iron toxicity tests were conducted with an alga (*Pseudokirchneriella subcapitata*), a cladoceran (*Ceriodaphnia dubia*), and the fathead minnow (*Pimephales promelas*) over varying dissolved organic carbon (DOC), hardness, and pH levels. The ability of DOC, hardness, and pH to explain variability in iron toxicity (EC10s) was evaluated using multiple linear regression (MLR) analysis. DOC and hardness were found to have an important influence on iron EC10s for *C. dubia*, while DOC, hardness, and pH all have an important influence on iron EC10s for *P. subcapitata* and *P. promelas*. Differences in the MLR models for each species demonstrate that the most sensitive of these three species varies depending on the water chemistry. For example, *P. promelas* is the most sensitive species in waters with very low pH (5.5-6), low hardness (10-20 mg/L), and low DOC (1 mg/L), but quickly becomes the least sensitive species as these parameters increase. *C. dubia* is the most sensitive species over the broadest range of DOC, hardness, and pH levels, but *P. subcapitata* becomes the most sensitive species in waters with a combination of low DOC and high hardness. This study clearly shows that water chemistry has an important influence on chronic iron toxicity and that the influence of water chemistry on iron toxicity is variable between the three species tested. From a regulatory perspective, the MLR models can be used to derive freshwater quality guidelines or criteria that account for site-specific chemistry.

#### 499 Water effect ratio model to support site-specific copper criteria across multiple drainages in an arid landscape

B. Fulton, ARCADIS / Risk Assessment and Ecological Sciences; D. Edge, A. Thatcher, J. Meyer, ARCADIS. Aquatic life criteria for copper are usually based exclusively on site-specific water hardness. However, previous studies have demonstrated other water quality parameters have equal or greater effects on copper toxicity than hardness alone. These water chemistry parameters should therefore be incorporated in the development of site-specific copper criteria to reflect mechanisms of copper toxicity and to provide an appropriate level of protectiveness. The water effect ratio (WER) procedure can be used to derive site-specific metal criteria that explicitly account for water chemistry. However, the available WER guidance is largely designed for point-source copper discharges to perennial systems. We present a case study that describes challenges associated with applying the WER procedure to a complex network of ephemeral drainages influenced by diffuse copper contamination in an arid landscape. To expand the application of the WER procedure to these ephemeral systems, we describe a WER model based on multiple regression analyses between empirical toxicity tests and water chemistries.

Hardness, alkalinity, organic carbon and total dissolved solids significantly correlated with site-specific copper toxicity. These relationships were used to develop a WER model that can be applied to a network of ephemeral waters at a mine site in the southwestern United States to derive adjusted copper criteria. The relationships between mechanisms of copper toxicity and model parameters are discussed, and a comparison to biotic ligand model predictions is included.

#### 500 Are Current Bioaccumulation Criteria and Approaches Relevant for Ionizing Substances such as Pharmaceuticals?

M.A. Bonnell, Environment Canada / New Substances Division, Environment Canada / Science and Risk Assessment Directorate. PB(T) properties of substances are most often used to "rank" or prioritize chemicals for further evaluation (e.g., Canadian categorization of the DSL), but can also be used to determine the "risk" an individual substance may pose to the environment without having extensive information on distribution or exposure (cf., listing in Annex XIII of REACH guidance). The basis for each of these properties can be linked to the assessment of legacy neutral organic chemicals that are often, but not always, of global concern (e.g., UNEP POPs). Current bioaccumulation criteria, assessment approaches and models have relied on the passive diffusion hydrophobicity-lipophilicity paradigm for the purpose of determining the potential for adverse effects from exposure to contaminants. We will examine the purpose of current bioaccumulation criteria and the extent to which these criteria and approaches should be applied to pharmaceuticals as example ionizing bio-active substances. We will also examine additional bioaccumulation metrics that could be used in place of or in addition to current criteria or approaches to bioaccumulation assessment.

#### 501 Prospects and limitations for applying mechanistic bioaccumulation models for ionogenic organic chemicals in aquatic ecosystems

J. Armitage, University of Toronto Scarborough; J.A. Arnot, ARC Arnot Research & Consulting Inc., University of Toronto Scarborough / Department of Physical and Environmental Sciences; T.N. Brown, Helmholtz Centre for Environmental Research UFZ / Department of Analytical Environmental Chemistry; F. Wania, University of Toronto at Scarborough / Dept. of Physical & Enviro. Science; D. Mackay, Trent University / Environmental Studies. Relatively few measured bioconcentration factors (BCFs) for ionogenic organic chemicals (IOCs) in fish are available and generalized mechanistic BCF models have recently been developed. While BCFs may be considered for categorization purposes (i.e. 'B' or 'Not B'), BCFs are generally not recommended for exposure and risk assessment because i) dietary exposure and food web dynamics are not considered and ii) pertinent environmental conditions in the field (e.g. pH, temperature, concentration of suspended solids) may differ substantially from those in the laboratory. Mechanistic bioaccumulation models that address differences in exposure and environmental conditions have been developed for neutral organic chemicals, providing a conceptual framework to follow when developing food web models for IOCs. The main questions with respect to modeling bioaccumulation of IOCs are i) how does ionization influence bioavailability of IOCs in the physical environment, ii) what is the influence of degree of ionization on chemical uptake efficiency (e.g. across the gills, in the gut) and iii) how does ionization influence the sorption capacity (i.e. distribution coefficients) of the organism. Here we further develop and apply a mechanistic BCF model for IOCs in fish to quantitatively examine these issues for sets of hypothetical IOCs. For example, we compare modeled BCFs and bioaccumulation factors (BAFs) in aqueous environments with different pH and suspended solid loads. We also explore the propagation of uncertainty in estimated biotransformation rate constants on the modelled BCFs and BAFs. Key strengths and uncertainties in the modeling approaches are identified and discussed in order to prioritize research needs.

#### 502 The role of membrane-water partitioning and membrane permeability of charged species for bioaccumulation of ionizable organic chemicals

B.I. Escher, Entox, The University of Queensland / National Research Centre for Environmental Toxicology. Predictive models for bioaccumulation are largely based on correlations with hydrophobicity parameters like the octanol-water partition coefficient. They fail for compounds with ionizable groups, metabolic capacity and those compounds whose uptake is driven by other processes than passive diffusion. A mechanistic understanding of the processes underlying bioaccumulation will improve the predictive power of bioaccumulation models. In this presentation we will give an overview on the relevant processes with a focus on ionizable compounds,



namely organic acids and bases, and how these can be described, experimentally determined and/or modelled. Membrane-water partitioning provides an appropriate surrogate for understanding the steady-state BCF of ionizable organic chemicals. Membrane permeation investigated with simplified test systems such as those that are used in the screening of pharmaceuticals will provide kinetic parameters for uptake of ionizable chemicals, including ion-trapping models, where it is assumed that only the neutral species can readily permeate a biological membrane. Apparently higher prediction of uptake than predicted from the pH-partition rule is then possible if ionic species will reconstitute inside the cell according to intracellular pH.

**503 Immobilised artificial membrane (IAM) chromatography: An investigation of the effect of pH on ionisable species** S. O'Connor, S. Latvala, M. Ledbetter, G. Hodges, S. Gutsell, Unilever; T. Gouin, Unilever / Safety and Environmental Assurance Centre. The octanol/ water partition coefficient is used in many areas of ecotoxicology and modelling as a measure of hydrophobicity or lipophilicity. It is well known that for ionisable chemicals or surfactants the measurement of  $K_{ow}$  is difficult due to the tendency of these types of materials to sit on the octanol/water barrier. Similarly, the accuracy of the prediction of  $K_{ow}$  for these types of materials is variable depending on the model used to calculate it. In part this is due to differences between  $K_{ow}$  and partitioning across biological membranes. It is possible that improved understanding of the intermolecular forces involved in membrane partitioning may be gained if more biologically relevant parameters are considered. Phosphatidylcholine (PC) is a ubiquitous phospholipid which is a major component of biological membranes. Immobilised Artificial Membrane (IAM) Chromatography utilises a phospholipid membrane made of PC. Using PC as a stationary phase and aqueous or organically modified buffers as a mobile phase, a partition coefficient ( $k_{IAM}$ ) for chemicals can be determined as a measure of the ability of a chemical to partition into the liposome layer (phospholipophilicity). As in biological systems and in buffers, ionisable compounds exist in different states which means that they interact to a different extent with biological membranes. This study was designed to determine the effect of the pH of the system on the retention of the chemicals in their ionic and neutral forms using IAM chromatography. As expected, the data indicated that the retention of ionisable chemicals is affected by the pH. Chemicals had a value for the neutral form ( $\log k_{IAM}$ ) and the ionised form, ( $\log k_{IAM (ion)}$ ) under relevant extreme pH conditions. However, at pH between these extremes, retention of the chemicals were based on an aggregate of both neutral and ionised species. It was also recorded that this aggregate retention indicated that the extent of ionisation of the system was not directly linked to the pKa of the compound under aqueous conditions but was shifted positively along the pH scale for acids and negatively for bases. The data also indicated that functional groups attached to the chemical also had an effect on both the difference between  $\log k_{IAM}$  and  $\log -k_{IAM(ion)}$  ( $\Delta k_{IAM}$ ) and the change in pKa ( $\Delta pKa$ ).

**504 Regulatory approaches to assessing Bioaccumulation of Pharmaceuticals: A Salty Tale** G. Rattray, Health Canada / Environmental Impact Initiative Division. Traditional regulatory approaches to determining whether a compound is bioaccumulative involve assessing partitioning between water and a lipid substitute, either through modelling or measuring ( $\log K_{ow}$ ), and subsequent evaluation against established criteria. While this approach may work well for neutral organics, in the case of pharmaceuticals where the active ingredient is often ionisable, the appropriateness of both the models and test methods are called into question. Furthermore, unlike neutral compounds where uptake is generally by passive diffusion, pharmaceuticals have a variety of routes into cells. This talk will explore the challenges of accurately assessing the bioaccumulation of pharmaceuticals in the context of an environment risk assessment conducted with a standardised regulatory set of data elements. The question of whether there is an opportunity to leverage some of the human health data (i.e. ADME) in a weight of evidence approach to get a more global view of the potential concern of the substance rather than simply assessing against arbitrary pass-fail criteria will be addressed.

**505 Development of An Initial Predictive Understanding of Bioconcentration of the Weakly Basic Pharmaceutical Diphenhydramine in Fish** B. Du, Baylor University / The Institute of Ecological, Earth, Environmental Sciences, Baylor University / Department of Chemistry and Biochemistry; Department of Environmental Science, Baylor, Baylor University / The Institute of Ecological, Earth, Environmental Sciences, Baylor; B.W. Brooks,

Baylor University / Environmental Science and Biomedical Studies, Baylor University / Department of Environmental Science; J. Berninger, Baylor University / Department of Environmental Science; K.A. Connors, Baylor University / Institute of Biomedical Studies, Baylor University / Department of Environmental Science; C.K. Chambliss, Baylor University; R.J. Erickson, U.S. EPA / Mid-Continent Ecology Division; J.W. Nichols, U.S. Environmental Protection Agency / ORD, NHEERL, Mid-Continent Ecology Division. Increasing evidence demonstrates that introduction of pharmaceuticals to aquatic ecosystems can result in the accumulation of parent compounds and/or their pharmacologically active metabolites in aquatic organisms. Unlike many historical contaminants, such as polychlorinated biphenyls and organochlorine pesticides, most therapeutics are ionizable compounds (~70% are weak bases), which suggests bioaccumulation and toxicity of these chemicals are influenced by site-specific pH. Unfortunately, the bioavailability and bioaccumulation of ionizable chemicals in general and pharmaceuticals in particular are poorly understood in fish and other aquatic organisms. The objective of present study was to develop an initial understanding of pH influences on the uptake of a weakly basic pharmaceutical, diphenhydramine (DPH), in the fathead minnow model. DPH, a first generation antihistamine drug found in many common over-the-counter formulations, was selected as a model compound because it is moderately lipophilic and ionized at environmentally relevant pH ( $pK_a$  -9.0,  $\log P$  -3.3). DPH has further been reported by our group to bioaccumulate in fish in the field, and to elicit pH dependent toxicity in laboratory studies with model aquatic organisms. We performed laboratory studies to characterize DPH uptake by adult male fathead minnows across an environmentally relevant pH gradient based on a slightly modified OECD guideline (TG305) for bioconcentration. Based on observations from these studies, we tested several models for predicting bioconcentration of ionizable compounds by fish. Finally, we examined the utility of these approaches for predicting bioaccumulation of DPH in an effluent-dominated stream, the North Bosque River, Texas, USA, during one of the most severe droughts in US history. Our findings indicate that accurate prediction of DPH uptake by fathead minnows requires a modeling approach that accounts for pH of the exposure water. Additionally, uptake of DPH by fish in the field was underestimated, suggesting that future studies are necessary to understand trophic transfer of ionizable pharmaceuticals in surface waters.

**506 Predicting the oral uptake efficiency of chemicals in mammals: Combining the hydrophilic and lipophilic range** I.A. O'Connor, M.A. Huijbregts, A.M. Ragas, J. Hendriks, Radboud University, Nijmegen / Environmental Science. Environmental risk assessment requires models for estimating the bioaccumulation of untested compounds. So far, bioaccumulation models have focused on hydrophobic compounds, and only a few have included hydrophilic compounds. Our aim was to develop a model to estimate the oral uptake efficiency of pollutants in mammals for compounds over a wide  $K_{ow}$  range with an emphasis on hydrophilic compounds. An existing model was extended by dividing the diffusion resistance through the membrane into a polar outer and apolar inner membrane resistance, which were related to the water phase using  $K_{ow}$  and the heptane water partition coefficient  $K_{hw}$ , respectively. The model was calibrated on two sets of data, i.e., the uptake efficiencies of environmental pollutants measured in different mammals during feeding studies and the human oral absorption efficiencies of pharmaceuticals. The new model estimated the uptake efficiency of neutral ( $RMSE=14.6$ ) and dissociating ( $RMSE=19.5$ ) compounds with  $\log K_{ow}$  ranging from -10 to +8. The inclusion of the  $K_{hw}$  improved uptake estimation for 33% of the hydrophilic compounds ( $r^2=0.60$ ,  $RMSE=21.2$ ) compared to the model version based on  $K_{ow}$  only ( $r^2=0.07$ ,  $RMSE=33.9$ ), while all hydrophobic compounds were estimated equally well by both model versions. Therefore, the new model provides a tool to estimate the uptake efficiency for new compounds such as pharmaceuticals and is based on mechanistic processes.

**507 Bioaccumulation of ionizing mono-phthalate esters in a marine aquatic food-web** EA Gobas, Simon Fraser University / School of Resource and Environmental Management (Faculty of Environment), Simon Fraser University / School of Resource & Environmental Management, Faculty of Environment, Simon Fraser University; M. McConnell, V. Otton, Simon Fraser University; M. Ikononou, Institute for Ocean Sciences. Dialkyl phthalate esters (DPEs) are a family of widely used industrial chemicals, which upon biotransformation and biodegradation, produce monoalkyl phthalate esters (MPEs), which due to their low  $pK_a$  of approximately 3.2

occur predominantly in the ionic phase at normal environmental pH levels. Due to their presence in aquatic environments, the bioaccumulation behavior of MPEs was studied in a range of aquatic species of a marine aquatic ecosystem in Vancouver. The results of the study indicate that the high solubility of MPEs in sea water causes a high bioavailability and a resulting high degree of biodegradation. The results further indicate that the high aqueous solubility and low lipophilicity of the ionic forms of MPE produces high respiratory elimination rates in aquatic biota and a lack of biomagnification or biodilution in the food-chain. Trophic Magnification Factors (TMFs) were approximately 1.0. Bioaccumulation Factors (BAFs) of MPEs were higher than expected from log D. This is likely due to the formation of MPEs resulting from the biotransformation of the DPEs. The bioaccumulation field study of MPEs reveals some key aspects of the bioaccumulation behavior of the anionic weak acids, which will be summarized and discussed in a modeling context.

**508 Ethinylestradiol disrupts fathead minnow (*Pimephales promelas*) population dynamics over three generations** A. Schwindt, Colorado State University / Fish, Wildlife, and Conservation Biology; D. Winkelman, Colorado Cooperative Fish and Wildlife Research Unit – USGS. Environmental estrogens are found worldwide in aquatic ecosystems and can lead to developmental and reproductive disruption in fishes. However, little is known about the population level consequences of exposure. Understanding population level responses to estrogen exposure is critical to the conservation and management of a wide variety of fishes. We evaluated the effects of 17 $\alpha$ -ethinylestradiol (EE2), the synthetic estrogen in human birth control, on three generations of fathead minnows (*Pimephales promelas*) in outdoor aquatic mesocosms and laboratory aquaria. We introduced five male and five female fish to each of 28, 1100L mesocosms and exposed the fish to 0, 5 (3.7), 10 (6.9), or 20 (15.2) ng/L of EE2 (measured concentrations) for 3.5 months (N=7). Fish were allowed to behave naturally and reproductive output was recorded. Following exposures in the mesocosms, the F1 offspring were counted, moved into the laboratory, and split into two groups. One group continued exposure to EE2 at nominal 5 or 10ng/L (N=4) while the other group recovered for 4 months (N=4). Breeding trials were conducted on the F1 for 1.5 months and reproductive output was recorded. Results from the mesocosms indicate that EE2 reduced both F0 reproductive output and F0 male survival. Results from the laboratory experiment indicate that early life EE2 exposure caused permanent reduction in F1 reproductive output evidenced by reduced numbers of F2 fish. In the F1 fish that were continuously exposed to EE2 no F2 offspring were produced and EE2 induced testis-ova in the gonad of F1 males. Our results suggest that exposure to trace concentrations of EE2 significantly impacts fathead minnow population dynamics and early life exposure causes irreversible damage to their reproductive system.

**509 G-Protein-Coupled Estrogen Receptor-1 (GPER): A Potential Target for Xenoestrogens During Vertebrate Embryogenesis** S. Jayasinghe, University of South Carolina / Department of Environmental Health Sciences; D.C. Volz, University of South Carolina, University of South Carolina / Department of Environmental Health Sciences. G-protein-coupled estrogen receptor 1 (GPER) is a G-protein-coupled receptor that induces non-genomic signaling in response to some nuclear estrogen receptor ligands. To date, the distribution and functional characterization of GPER within vertebrate organs have been restricted to juvenile and adult animals. However, the role of GPER during vertebrate embryogenesis is currently unknown. The overall objectives of this project were to (1) characterize the spatiotemporal expression patterns of *gper* during vertebrate embryogenesis and (2) identify the potential impacts of ligand-induced GPER activation during vertebrate embryogenesis. Using wildtype zebrafish, we first demonstrated that (1) *gper* exhibited strong stage-dependent expression patterns during embryogenesis; (2) aberrant GPER activation by a selective GPER agonist (G-1) – but not inhibition by a selective GPER antagonist (G-15) – resulted in developmental malformations; and (3) co-exposure with G-15 blocked G-1-induced teratogenesis, suggesting that G-1 toxicity is mediated via aberrant GPER activation. Based on these data, we hypothesized that the developing cardiovascular system was the primary target organ for G-1-induced toxicity. Using quantitative *in vivo* imaging assays and transgenic zebrafish (*flil:egfp*), we then demonstrated that G-1 exposure resulted in impaired cardiac morphology and performance as well as, at higher concentrations, early circulatory arrest. Similarly, embryonic exposure to reference xenoestrogens – genistein and bisphenol-A (BPA) – with known affinity

to human GPER resulted in concentration-dependent effects (albeit with lower potency than G-1) on the developing cardiovascular system. To begin revealing mechanisms of G-1-, genistein-, and BPA-induced cardiovascular malformations, we then relied on a battery of inhibitors and antagonists that target signaling events downstream of GPER. While genistein and BPA appeared to involve distinct signaling mechanisms compared to G-1, our data suggest that cardiovascular malformations resulting from G-1, genistein, or BPA exposure likely involved EGFR transactivation, MAPK activation, and reduced levels of vascular endothelial growth factor- $\alpha$  (Vegf- $\alpha$ ) and Notch ligand delta like 4 (Dll-4) mRNA. Overall, our findings suggest that GPER activation represents a potentially novel and understudied mechanism of cardiovascular toxicity for environmentally relevant chemicals that affect vertebrate embryogenesis.

**510 Towards better understanding of the mechanisms of action of the ureic based herbicide linuron** C.J. Martyniuk, University of New Brunswick / Dept. of Biology/Canadian Rivers Institute, University of New Brunswick / Biology; A. Ornostay, University of New Brunswick Saint John / Department of Biology and Canadian Rivers Institute, University of New Brunswick Saint John / Biology; S. Alvarez, Donald Dansforth Plant Sciences Center; A. Cowie, M. Hindle, C. Baker, University of New Brunswick; N. Hogan, University of Saskatchewan; B. Lo, Nautilus Environmental; C. Kennedy, Simon Fraser University; J. Elphick, V.L. Marlatt, Nautilus Environmental. Linuron (LIN), a ureic-based herbicide, is an endocrine disruptor with a suspected anti-androgenic mode of action (MOA). However, the effects of this herbicide on the teleostean reproductive axis are not completely understood. We have measured responses in both male and female fathead minnows (FHM) to LIN using reproductive endpoints that encompass a range of biological organization (gene expression, proteomics, sex steroids, vitellogenin, gonadal histopathology, secondary sex characteristics, fecundity, and survival). Using an *in vitro* steroid production bioassay, we show that LIN does not significantly affect  $E_2$  production in a 12 hr incubation period, however LIN did affect molecular signaling pathways that included the biosynthesis of cholesterol, T-cell receptor signaling, and increased the gene expression networks of follistatin and steroid hormone receptors. Comparisons of expression patterns and support vector machine classification demonstrated that the gene expression responses to LIN are more similar to that activated by anti-androgens such as flutamide when compared to androgens. A 21d reproductive bioassay using 1, 10, and 100  $\mu$ g LIN/L showed that secondary sex characteristics, fecundity, and survival of fry were not affected by LIN. There were also no significant changes in ovary histology or mRNA abundance for steroid-related genes (e.g. *aromatase*, *estrogen receptors*). However, plasma vitellogenin (Vtg) production was significantly decreased in females after exposure to 1 and 100  $\mu$ g LIN/L, suggesting reproductive impairment may be occurring at the liver and Vtg synthesis. There was no Vtg induction in males. Using iTRAQ quantitative proteomics, LIN altered many proteins involved in stress and cell signaling (e.g. prostaglandin E synthase 3, cell death proteins, glutathione S transferases). Sub-network enrichment analysis identified that interferon and epidermal growth factor signaling were regulated by LIN, and these signaling pathways were associated with depressed plasma vitellogenin in females. These data suggest that LIN can have adverse effects at the site of the liver and disrupt gene pathways in the ovary that have the potential to impact steroid production.

**511 Impacts of hypersaline acclimation on the sublethal toxicity of chlorpyrifos on salmonid olfaction** L.A. Maryoung, University of California – Riverside / Environmental Science, University of California Riverside / Department of Environmental Sciences; N. Agarwal, G. Hardiman, R. Sasik, University of California, San Diego / Department of Medicine; K. Tierney, University of Alberta / Biological Sciences; D. Schlenk, University of California-Riverside / Department of Environmental Sciences. Acclimation to hypersaline environments has been shown in previous studies to enhance the acute toxicity of organophosphate pesticides to euryhaline fish species; however sublethal effects have been far less studied. The present study focuses on the sublethal toxicity of the organophosphate chlorpyrifos to salmonid olfaction during and after hypersaline acclimation. Salmonids rely on olfaction to detect chemical cues that provide crucial information about food, predators, reproductive status of mates, environmental contamination, and natal streams of imprinting. The purpose of this study was to link molecular, physiological, and behavioral alterations in salmonids undergoing exposure to chlorpyrifos while acclimating to hypersaline conditions.



To determine molecular effects of combined exposure, salmonids were acclimated to three different salinities (8ppt, 16ppt, 32ppt) for one week and mRNA was collected from the olfactory rosettes. Microarray hybridization was used to determine differences in gene expression for the different salinity treatments. Through literature searches potential target genes involved in signal transduction, which have been shown to be impacted by chlorpyrifos exposure in zebrafish, were identified and targeted in microarrays. The genes included gefitin, parvalbumin 8, calmodulin 2b, chloride intracellular channel 4, guanine nucleotide binding protein (G protein) gamma 1 subunit, and adrenergic alpha 2B receptor. To assess physiological effects, electroolfactograms and behavioural assays will be compared to molecular effects to determine appropriate and relevant adverse outcome pathways for salmonids undergoing exposure during saltwater acclimation (NIEHS P30ES07033).

**512 Dietary selenomethionine exposure alters aerobic metabolism and methionine catabolism in adult zebrafish** J. Kallarakavumkal Thomas, University of Saskatchewan / Toxicology Centre, University of Saskatchewan / graduate student; S.B. Wiseman, University of Saskatchewan / Toxicology Centre, University of Saskatchewan / Post-Doctoral Fellow; J.P. Giesy, University of Saskatchewan / Biomedical Veterinary Sciences & Toxicology Centre, Michigan State University / Department of Animal Science, University of Saskatchewan / Toxicology Centre; D. Janz, University of Saskatchewan / Toxicology Centre, University of Saskatchewan / Veterinary Biomedical Sciences. Our previous study reported impaired swimming and increased accumulation of stored energy in adult zebrafish (*Danio rerio*) exposed chronically to environmentally relevant concentrations of dietary selenomethionine (SeMet). The present study investigated potential mechanisms of impaired swimming and elevated stored energy accumulation in adult zebrafish exposed to similar concentrations of dietary SeMet. Fish were fed 1.3, 3.4, 9.8 and 27.5 µg Se/g, dry weight in the form of SeMet for 90 days. At the end of the exposure, fish from each treatment group were divided into three subgroups: a) no swim, b) swim, and c) repeat swim. Fish from the no swim group were euthanized immediately at 90 days and whole body triglycerides, glycogen and lactate, and gene expression of energy metabolism and methionine catabolism enzymes were determined. Individual fish from the swim group were placed in a swim tunnel respirometer and swimming performance was determined using the critical swimming speed ( $U_{crit}$ ) method. After both  $U_{crit}$  and oxygen consumption ( $MO_2$ ) analyses, fish were euthanized and whole body energy stores and lactate were determined. Similarly, individual fish from the repeat swim group were subjected to two  $U_{crit}$  tests ( $U_{crit-1}$  and  $U_{crit-2}$ ) performed with a 60 min recovery period between tests, followed by determination of energy stores and lactate. Impaired swim performance was observed in fish fed greater than 3 µg Se/g in the form of SeMet. Oxygen consumption, standard metabolic rate (SMR) and cost of transport (COT) were significantly increased, and factorial aerobic scope (F-AS) was significantly reduced in fish fed elevated dietary SeMet. Whole body triglycerides increased with increasing dietary SeMet exposure. Both dietary SeMet exposure and swimming had significant effects on whole body lactate concentration in fish. Elevated SeMet exposure significantly down-regulated mRNA abundance of protein tyrosine phosphatase 1B (PTP 1B) in muscle, and  $\beta$ -hydroxyacyl coenzyme A dehydrogenase (HOAD), sterol regulatory element binding protein 1 (SREBP 1) and methionine adenosyltransferase 1 alpha (MAT 1A) in liver of adult zebrafish. Ongoing work is investigating muscle histology of adult zebrafish fed elevated dietary SeMet. Overall, the results of this study provide new insights into mechanisms of SeMet toxicity to freshwater fish.

**513 Arsenic reveals molecular mechanism, physiological effects, population-level variation, and evolutionary basis for osmotic plasticity in killifish** J.R. Shaw, Dartmouth College / The School of Public and Environmental Affairs and The Center for Genomics and Bioinformatics; T.H. Hampton, Dartmouth Medical School; B.L. King, Mount Desert Island Biological Laboratory; A. Whitehead, University of California, Davis; R.N. Keith, Indiana University / The School of Public and Environmental Affairs and The Center for Genomics and Bioinformatics; E.G. Notch, D. Jung, Dartmouth Medical School; S.P. Glaholt, Indiana University / The School of Public and Environmental Affairs and The Center for Genomics and Bioinformatics; J.K. Colbourne, Indiana University / The Center for Genomics and Bioinformatics; C.Y. Chen, Dartmouth College; B.A. Stanton, Dartmouth Medical School. *Fundulus heteroclitus* is a teleost adapted to life in estuaries. Its tidal niche produces large osmotic gradients; and many populations tolerate abrupt changes in salinity along this gradient, ranging

from freshwater to marine. Killifish also reside in close proximity to humans, increasing their risk of exposure to anthropogenic stressors. Since killifish provide unique access to the biology that underlies physiological plasticity and stress tolerance, we sequenced, assembled, and annotated its transcriptome, expressed under 72 different conditions. This sequence information was used to discover how arsenic disrupts osmotic plasticity, investigate the response across populations and explore underlying evolutionary mechanisms. We observed that non-toxic arsenic concentrations disrupt chloride balance and block seawater acclimation. Microarray experiments conducted as fish are moved from freshwater to seawater demonstrate that the combined stresses of arsenic and salinity produces large, non-additive antagonistic, interactions at the mRNA level that are consistent with the phenotypic observation that arsenic constrains physiological plasticity by inhibiting seawater acclimation. These interaction genes, which functionally are enriched in biological processes such as mitochondrial function, and tight junction signaling, are more tightly regulated between individual fish than other differentially regulated genes across all treatments (i.e. canalized). In experiments of three natural populations of killifish residing along a salinity cline ranging from freshwater to marine environments, we observed salinity dependent variation in the expression and regulation of these arsenic and seawater interaction genes. These genes are locally enriched and more tightly regulated in only the freshwater population. To address evolutionary mechanisms for the tight regulation, we observed that interaction gene sets have fewer associations with upstream controlling genes. This suggests that canalization has evolved via a more loosely coupled association with regulating genes as a means to reduce expression noise in variable environments. Collectively, these studies demonstrate the power of genomics applied to the killifish system to understand environmental effects on genes in natural populations.

**514 Linking the sublethal effects of mercury on the hypothalamic-pituitary-gonadal axis of yellow perch in Lake Michigan to ecological effects** C.A. Murphy, Michigan State University / Department of Fisheries and Wildlife, Lyman Briggs College, Michigan State University / Lyman Briggs College; B. Armstrong, Michigan State University; M. Carvan, R. Goetz, University of Wisconsin, Milwaukee; N. Basu, J. Head, University of Michigan. The development of appropriate extrapolation models to link key molecular initiating events to adverse outcomes is a crucial component of the adverse outcome pathway (AOP) framework. Only recently, a few of these models, that convert sublethal responses of stressors to endpoints that can be interpreted at the ecological level, have been developed – and only in a few model species, eg fathead minnow. Very few extrapolation models exist for larger, long-lived, commercially valuable species. Here we illustrate how this can be done on a species of commercial and ecological relevance to the Great Lakes, yellow perch (*Perca flavescens*). We incorporate the sublethal effects of ecologically relevant doses of mercury contamination on the Hypothalamus-Pituitary-Gonadal (HPG) axis of yellow perch, measured at the molecular and physiological level, and link these effects to population relevant points such as fecundity. Such endpoints can then be input into population models to project population effects. In our approach, we used laboratory fish exposed to mercury in a controlled experiment that spanned the period of gonadal growth. We sampled fish at several time points during this experiment to build and calibrate a vitellogenesis model. We then linked the vitellogenesis model to perch population models – in these models, we attempt to incorporate differences that manifest as a result of differences in life history within and between species. We collected fish from various sites around Lake Michigan to determine the extent of mercury contamination and how much each population/sub population is likely impacted. We found that different populations of yellow perch are exposed to different levels of mercury contamination – such exposure manifests in measurable sublethal effects that can be incorporated into our models. Our model results suggest that some perch populations are more impacted and are more at risk than others due to mercury contamination and that this approach may be useful to determine the harmful effects of sublethal effects across a wider array of populations and species.

**515 Mechanistic Insight into the Effect of Venlafaxine on Cortisol Stress Axis Activity in Rainbow Trout** M. Vijayan, University of Waterloo / Biology; N. Melnyk-Lamont, University of Waterloo; C. Best, M. Rodriguez, University of Waterloo / Biology. Venlafaxine, a serotonin-norepinephrine reuptake inhibitor, is a commonly prescribed antidepressant. Although this drug is present in municipal waste water effluents, its impact on fish



performance is largely unknown. We carried out a series of studies to test the hypothesis that environmentally relevant levels of venlafaxine disrupt the functioning of the hypothalamus-pituitary-interrenal (HPI) axis in rainbow trout (*Oncorhynchus mykiss*). Fish were exposed to environmentally relevant venlafaxine concentrations (0.2 µg/L and 1.0 µg/L) and sampled 7 d after exposure. Fish were also subjected to a physical stressor to assess the impact of the drug on the HPI axis performance. In the brain, venlafaxine treatment impacted the levels of neurotransmitters (serotonin, norepinephrine, dopamine) and their metabolites in a region-specific manner. Also, the drug disrupted mRNA abundances of genes encoding key proteins involved in HPI axis activity, including corticotropin-releasing factor, pro-opiomelanocortin and steroidogenic acute regulatory protein in selected brain regions. At the peripheral level, the stressor-induced glucose response was attenuated by venlafaxine treatment. The drug also disrupted the transcript levels of key corticosteroidogenic genes in the head kidney post-acute stressor exposure. Altogether, environmentally relevant levels of venlafaxine perturbs the highly conserved stress axis activation in rainbow trout, and the mode of action involves the disruption of key central and peripheral targets involved in HPI axis functioning. This study was supported by an NSERC discovery grant.

**516 Studying naphthenic acid toxicity using the model green algae *Chlamydomonas reinhardtii*** K.L. Goff, University of Saskatchewan / Toxicology; J. Headley, Environment Canada; J.R. Lawrence, Water Science and Technology Directorate, Environment Canada / Water Science and Technology Directorate, Environment Canada / Research Scientist; T. Ellis, Canadian Light Source. Studies of the toxicity and biodegradation of oil sands naphthenic acid fraction components (NAFCs) have chiefly focused on bacteria, fungi, and emergent macrophytes, with a large and still-growing body of information on animal toxicity. This complex and still poorly quantified mixture is known to have toxic effects on diverse taxonomic groups, including fish, benthic invertebrates, birds, small mammals, emergent macrophytes, and has been identified as a possible primary agent of toxicity in the oil sands process water mixture. The mode of toxic action is not known, though classical naphthenic acids are anionic surfactants and in some species their surfactant properties may play a role in their ecotoxicology. One of the algal species identified in oil sands process waters is *Chlamydomonas frigida*; while there is little information in the literature regarding this species, the genus is well-understood due to *C. reinhardtii*'s frequent use as a model organism. Three lines of *C. reinhardtii* were selected for inclusion in this study; an extremely well-characterized laboratory wild-type strain, and two mutants lacking cell walls as initial trials indicated the possibility of NAFC interactions with the cell wall. All three were grown heterotrophically in nutrient media spiked with a dilution series of NA. Culture densities were measured to obtain growth curves and survival data, and examined for changes in growth form. Exposed cells were examined with infrared spectromicroscopy, confocal laser scanning microscopy, and subjected to lipid analysis. The NAFC-containing media was also analyzed using high resolution mass spectrometry for algae-mediated changes in NAFC composition. Tolerance and growth data will be presented for each line, and changes in algal physiology and NAFC composition of the media following algal growth will be reported.

**517 Evaluating the Effects of Naphthenic Acid Fractions on *Oryzias latipes*** A.E. Bauer, University of Waterloo / Department of Biology; R.A. Frank, Environment Canada / AEPRD, Environment Canada / Dept. of Env. Biology & Toxicology, University of Guelph / Dept. of Env. Biology & Toxicology; J. Headley, K. Peru, Environment Canada; M. Hewitt, Environment Canada / National Water Research Institute; A. Farwell, University of Waterloo / Department of Biology; D.G. Dixon, University of Waterloo. Naphthenic Acids (NAs) are a complex group of alkyl-substituted acyclic, monocyclic and polycyclic carboxylic acids, with surfactant properties that are released from oil sands during bitumen extraction. In fact, they have been deemed the most toxic component of oil sands process material and process water (OSPW). Studies have indicated that an increase in NA concentration within reclamation pond water increases the potential for detrimental reproductive effects, as well as increased larval mortality and pathologies in fish. High molecular weight NAs are degraded more slowly than low molecular weight NAs resulting in a higher proportion of high molecular weight NAs in aged OSPW. Furthermore, studies have identified a decrease in toxicity associated with aged OSPW and degraded NAs. This has led to the thought that not all compounds within these classes are of equal toxicity. This area is where research is lacking. Few studies have evaluated

the toxicity associated with a fractionated extract of oil sands NAs. This study assesses the difference in toxicity associated with different molecular weight fractions of an oil sands NA extract on Japanese medaka (*Oryzias latipes*). The NA extract was fractionated by distillation, allowing for the collection of fractions by molecular weight. Each fraction was then tested at multiple concentrations with an acute embryo-larval bioassay to determine 10d LC50s, deformities, and hatch endpoints. These endpoints were then compared between fractions. Results suggest that not all fractions display equal toxicity and that toxicity may be associated with molecular weight. Furthermore, this study has identified time to hatch and hatch success as relevant endpoints in relating NA molecular weight to its toxicity.

**518 Evaluation of the effects of oil sands-influenced surface waters on the health of fishes** M.R. Van den Heuvel, Canadian Rivers Institute, University of Prince Edward Island; N.S. Hogan, University of Saskatchewan / Toxicology Centre. Since 1995, efforts have been underway to determine the impacts of oil-sand related contamination on fish health. The overarching questions are, 1) do toxicological effects occur? 2) what are the causative agents? and 3) what are the thresholds of effect? Initial studies with yellow perch showed no reproductive effects, but indications of increased disease. This led to the present research program (2008-present) to identify disease and causes as well as to follow up on reproductive and multi-species responses. The experimental program used stocking studies with wild yellow perch and white sucker into experimental ponds in conjunction with in situ trout caging studies, and rainbow trout laboratory studies with oil-sands derived naphthenic acids. Field/in situ research was conducted with waters from three primary sites: Mildred Lake, a reservoir receiving only Athabasca River water, used as the reference site; South Bison Pond, a drainage basin that has received unextracted oil sands-contaminated overburden; and Demonstration Pond, a constructed pond containing oil sands mature fine tailings capped with fresh water. The primary results showed consistent disease responses in yellow perch, but limited effects in white sucker. Male yellow perch and white sucker showed lower testicular development in the most contaminated site. Trout in situ studies also showed suppressed immune function and disease. Elevated bile fluorescence and induced CYP1A enzymes were consistent in all species. Injection studies with naphthenic acids in laboratory trout were not consistent with the effects observed in the field. Evaluation of water chemistry and exposure indicators suggest that the observed effects are due to organic compounds and not salinity. Fish are exposed to both naphthenic acids and polycyclic aromatic hydrocarbons and the involvement of PAHs in the immune response is favored. While organic compounds likely contribute to effects, alkalinity/pH could also become a limiting factor in fish survival. Without specific causative agents, thresholds cannot be determined; however, based on measurement of bulk naphthenic acids, effects on disease will begin to occur at 5 mg/L, and effects on reproduction and disease are likely above 10 mg/L of naphthenic acids in yellow perch, the most sensitive species examined to date.

**519 Assessment of immunotoxicity of oil sands process water in animals** M.O. Hagen, E. Garcia-Garcia, University of Alberta / Biological Sciences; A. Oladiran, B.A. Katzenback, S. Mitchell, M. Karpman, University of Alberta; M. Gamal El-Din, University of Alberta / Civil and Environmental Engineering; J. Martin, University of Alberta; M. Belosevic, University of Alberta / Biological Sciences. In these studies, we exposed goldfish OSPW using a real-time flow-through apparatus for up to 12 weeks and mice orally to the organic fraction of OSPW (OSPW-OF). For studies using fish, we measured gene expression of pro-inflammatory cytokines in the gill, kidney, and spleen using quantitative-PCR. We observed an up-regulation of cytokine gene expression after acute exposure of fish to either high concentrations of commercial NAs or aged OSPW. Sub-chronic exposure of fish to high concentrations of NAs caused a down-regulation in immune gene expression in fish exposed. In order to determine whether goldfish host defense was affected by exposure to NAs, fish were infected with a blood parasite *Trypanosoma carassii* and their ability to control the infection was documented. Following acute exposure to NAs fish exhibited consistently lower parasitemia compared to controls. After sub-chronic exposure of fish to NAs, fish had higher parasitemia than control fish. Thus, the exposure of goldfish to NAs or aged OSPW elicited an enhancement in pro-inflammatory gene expression and increased resistance to parasitic infection after acute exposure, and down-regulation of immune gene expression and increased susceptibility to parasitic infection after sub-chronic exposure to NAs. In contrast to the results obtained in studies using fish, the immune gene

expression was significantly down-regulated in mice after acute exposure to OSPW-OF, and after sub-chronic exposure (8 weeks) the mRNA levels of various immune genes were similar to those of sham-inoculated controls. The immune gene expression differed between the organs examined (spleen, liver and mesenteric lymph nodes) during both acute and sub-chronic exposures of mice to OSPW-OF. We also developed a rapid in vitro assay for assessment of the immunotoxic effects of OSPW-OF.

**520 Assessing Deposition of Polycyclic Aromatic Compounds (PACs) in air across the Alberta Oil Sands Using Passive Air Samplers** T. Harner, Environment Canada / Atmospheric Science & Technology Directorate, Environment Canada / ASTD; K. Su, S. Genualdi, J. Karpowicz, C. Mihele, J. Charland, L. Zhang, Environment Canada. As part of the Joint Implementation Plan for Oil Sands Monitoring, polycyclic aromatic compounds (PACs) have been measured in air to track concentrations and associated deposition, with the ultimate objective of assessing cumulative environmental impacts. This study is responding to concerns regarding deposition of PACs to the Athabasca river and its watershed and potential health risks to humans and other terrestrial and aquatic life. Continuation of the air monitoring network in the short and long-term will provide a baseline against which the expansion of oil sands activities and future PAC air concentrations can be compared. The spatial component of the air monitoring study uses passive air samplers comprising polyurethane foam (PUF) disks housed in stainless steel chambers. These are deployed at 17 sites, some of them close to emission sources while others at varying distances from the active zones, over 2-month integration periods, since November 2010. Concurrently, active air samples are collected at three of the sites, every 6<sup>th</sup> day, for 24 hours, and provide a means of calibrating the passive samplers. The results of the calibration indicate that the PUF disks are able to capture both gas-phase and particle-phase PACs. Comparison of passive results against the high volume air sampler data for the three collocated sites indicates that the passive sampling rates for both gas- and particle-phase PACs is approximately 5 m<sup>3</sup>/day. These preliminary findings support the continued use of PUF disk samplers as a long-term monitoring tool in the oil sands region. The simplicity and small-size of the samplers coupled with their ability to operate electricity-free contributes to their feasibility. Air concentration results from the first year of sampling, targeting 16 PAHs and dibenzothiophene including their C1 to C4 alkylated homologues, will be presented. The use of the data in a model to assess atmospheric deposition of PACs across the entire oil sands region will also be discussed.

**521 Chemical fingerprinting of oil sands naphthenic acids from natural and industrial sources** R.A. Frank, Environment Canada / AEPRD, Environment Canada / Dept. of Env. Biology & Toxicology, University of Guelph / Dept. of Env. Biology & Toxicology; G. Bickerton, J. Headley, Environment Canada; S.J. Rowland, University of Plymouth / School of Geography, Earth and Environmental Sciences; K. Peru, J. Roy, Environment Canada; A. Scarlett, University of Plymouth / School of Earth, Ocean & Environmental Science, University of Plymouth / School of Geography, Earth and Environmental Sciences; M. Hewitt, Environment Canada / National Water Research Institute. The rapid expansion of oil sands development, especially within the past 10 years, has raised many concerns regarding the potential impacts on the environment and downstream communities. Extensive research is currently underway to identify a “chemical fingerprint” of oil sands compounds that will enable the differentiation of industrially and naturally derived oil sands materials. Recent advancements in chromatographic separations and high resolution mass spectrometry analyses are beginning to allow for the elucidation of specific classes of compounds as well as some individual structures contained within complex mixtures of oil sands organic acids. Between 2009 and 2011, samples collected from several sites within the Athabasca River system, containments of oil sands process-affected water (OSPW), groundwater interceptor well systems near basins containing OSPW, and groundwater wells and seeps outside of industrial leases were screened by synchronous fluorescence spectroscopy (SFS). A unique SFS fingerprint for a naphthenic acid extract (NAE) from fresh OSPW has been identified and subsequent analysis by GCxGC-TOF-MS indicated that classes of polycyclic acids with one or more aromatic rings are contributing to this fingerprint. SFS analysis of groundwater samples collected on industrial leases from interceptor well systems, as well as from groundwater wells outside of these collection systems, exhibited spectra consistent with NAE and OSPW. Although the majority of samples collected beyond industrial leases did not exhibit the SFS

fingerprint, several groundwater samples collected adjacent to OSPW containments displayed this profile. To examine the contributions of oil sands NA mixtures originating from both natural and lease sources further, selected samples were profiled by electrospray high resolution mass spectrometry and GCxGC-TOF-MS, with the intention to identify instrument-specific “chemical fingerprints”. Both techniques are proving to be complimentary, and allowing for the first differentiations between naturally and industrially derived oil sands naphthenic acids.

**522 New analytical strategies for monitoring of oil sands tailings ponds and natural waters in the lower Athabasca Region of Alberta, Canada** M.S. Ross, A. dos Santos Pereira, University of Alberta / Division of Analytical and Environmental Toxicology; J.W. Martin, Trent University / Division of Analytical and Environmental Toxicology. Oil sands process affected water (OSPW) is acutely and chronically toxic to aquatic and terrestrial organisms, but the chemicals responsible for the adverse effects are unknown. Furthermore, increasing concerns over potential seepage of OSPW to surface and groundwaters have lead to increased monitoring efforts in the Athabasca River watershed. Many of the primary constituents of OSPW, such as naphthenic acids, also occur naturally in bitumen and may enter receiving waters naturally through groundwater transport or erosion of exposed oil sands formations. A key question to be addressed in monitoring efforts is whether such constituents arise from natural processes, or due to oil sands development. Herein, we report on several new analytical strategies developed with the aim of characterizing the complex mixture of chemicals in OSPW, identifying key toxic components, or for differentiating sources in the environment. In the first study, a liquid chromatography-high resolution mass spectrometry method was used to fingerprint classical O<sub>2</sub>-naphthenic acids in surface and groundwater from the Athabasca Region. Downstream sampling sites were clearly discernable from upstream sites based on the increased proportion of bitumen derived acids, whereas groundwaters were identifiable based their higher percentage of unsaturated, or multi-cyclic, acids. Next, a comprehensive two-dimensional liquid chromatography method was developed, which provided greater chemical formula group-type separations than previously achievable by one-dimensional chromatography, and which also provides fingerprinting capabilities. Finally, ultrahigh resolution Orbitrap mass spectrometry was coupled to liquid chromatography, facilitating the discovery of several isomers and a novel non-naphthenic acid class of O<sub>2</sub> compounds in OSPW. These methods have increased our understanding of the chemical complexity of OSPW, and together add to the toolbox of methods that will be needed to explain the toxic effects of OSPW or unravel the sources of contaminants in surrounding natural waters.

**523 Comparison of extractable acids from Athabasca oil sands tailings ponds, interceptor wells and groundwaters using GCxGC-MS** A. Scarlett, University of Plymouth / School of Earth, Ocean & Environmental Science, University of Plymouth / School of Geography, Earth and Environmental Sciences; C.E. West, Plymouth University / SoGEES, University of Plymouth / Biogeochemistry Research Centre; S.J. Rowland, D. Jones, University of Plymouth / SoGEES. Recent analyses using tandem gas chromatography – mass spectrometry (GCxGC-MS) of oil sands process-affected waters (OSPW) from Alberta Canada has started to reveal some of chemical structures present in the acid-extractable fraction of these highly complex mixtures. Some of these compounds were demonstrated to have very unusual diamondoid acid structures and these may be of use for differentiation between environmental samples and tracking leakage from tailings ponds into groundwaters. More recent studies using “reverse phase”, i.e. polar primary with apolar secondary columns, GCxGC-MS have shown that as well as these alicyclic structures, the acid extractable fraction of OSPW (analysed as methyl esters) also contained a sizeable aromatic fraction. Some of the proposed structures of these aromatic acids have been modelled to be hormonally active and acutely toxic to fathead minnow (LC<sub>50</sub> ca 1 µM). These polycyclic monoaromatic structures may also provide a means by which environmental samples may be differentiated. In the present study, 14 environmental water samples were analysed by both normal phase and reverse phase GCxGC-MS. The water samples (5 ml) were collected from the Athabasca region of Alberta Canada, including tailing ponds from two oil sands extraction sites, interceptor wells, on-lease and off-lease groundwaters. Despite the very small quantities of water extracted, the analyses showed distinctive patterns in the 2D chromatograms. Individual resolved peaks with good mass spectra allowed comparison between the samples and revealed that some of the aromatic acids present in the OSPW acid extracts

were also present in the on-lease groundwaters and some of the off lease water samples. This current study has shown that analyses by GCxGC-MS can not only differentiate between environmental samples but can also track individual, potentially toxic, compounds.

**524 Weighing” the Evidence: Relevance and Transparency in the Evaluation of Endocrine Activity”** C. Borgert, Applied Pharmacology and Toxicology Inc.; E.M. Mihaich, Environmental and Regulatory Resources, LLC; L.S. Ortego, Bayer CropScience / Ecotoxicology, Bayer CropScience; S. Marty, The Dow Chemical Company; J. Brausch, F. Hess, BASF Corporation. There are several key elements to a scientifically based weight of evidence (WoE) process, including the development of a priori specific hypotheses, a systematic review of relevant literature including an evaluation of data quality and reliability, and an assessment of consistency, specificity and reproducibility of effects. These elements all focus on the relevance and transparency of the data and the assessment process. To this end, a conceptual framework for conducting weight of evidence (WoE) evaluations of endocrine screening and testing data (Borgert et al., 2011) was developed. The framework was designed to be broadly applicable, but with an emphasis on data emerging from the US EPA's Endocrine Disruptor Screening Program (EDSP), and specifically tailored to accommodate endpoints measured in the eleven Tier 1 Endocrine Screening Battery (ESB) assays. The framework calls for a transparent process whereby specific hypotheses are formulated and data quality is evaluated with respect to overarching scientific principles, including primary validity of the measurements, reliability of data reporting, and probative capability of the study design to evaluate causation. The framework also calls for assigning quantitative weighting factors to assay endpoints ( $W_{REL}$ ) that reflect their relevance for deciding specific hypotheses in relation to in vivo endocrine activity and effects. This is an important step in the WoE framework as it explicitly recognizes the fact that some assays are very specific and sensitive for evaluating certain hypotheses related to endocrine activity but may be irrelevant for evaluating others. For example, an increase in thyroid follicular hyperplasia, as assessed in the amphibian metamorphosis assay, would be expected to have a very high  $W_{REL}$  for evaluating the hypothesis that a chemical has potential thyroid antagonist activity, but would have a very low  $W_{REL}$  for evaluating the hypothesis that a substance has potential estrogen or androgen agonist or antagonist activity. Example derivations of rank ordered  $W_{REL}$  values for evaluating Tier 1 EDSP hypotheses will be presented for compounds tested in the validation phase of the EDSP, such as methoxychlor, flutamide and/or phenobarbital, with particular emphasis on endpoints measured in the fish screening and frog metamorphosis assays.

**525 ortho-Phenylphenol: Results of the Amphibian Metamorphosis Assay and the Fish Short Term Reproduction Assay** C. Lehman, The Dow Chemical Company, The Dow Chemical Company / Toxicology and Environmental Research and Consulting; K. Coady, R. Currie, T. Marino, J. Fiting, K. Hutchinson, D. Louch, N. Malowinski, L. McFadden, J. Thomas, The Dow Chemical Company / Toxicology and Environmental Research and Consulting. *ortho*-Phenylphenol (OPP), an antimicrobial agent found in many disinfectants, was evaluated for potential endocrine disrupting activity in both the Amphibian Metamorphosis Assay (AMA) and the Fish Short Term Reproduction Assay (FSTRA). In the AMA, tadpoles were exposed to several OPP concentrations in a continuous flow-through test system for either 7 or 21 days. In the FSTRA, fathead minnows were exposed to OPP under continuous flow-through conditions for 21 days. In the AMA, there were no significant differences observed on day 7. On day 21, tadpoles exposed to the highest OPP concentration showed a significant developmental delay; no other endpoints were statistically significant at this timepoint nor were there signs of asynchronous or advanced development. Compared to the thyroid glands from control organisms, there were no treatment-related histopathological effects observed in the thyroid glands from OPP-exposed tadpoles. Therefore, OPP is considered “likely thyroid inactive” in the Amphibian Metamorphosis Assay. In the FSTRA, there were no significant differences between control and OPP-exposed fish in regard to fertility, fecundity, male and female wet weight and length, male and female gonado-somatic indices, tubercle scores, or blood plasma concentrations of VTG in male or female fish. Except for a single male fish from the highest OPP concentration with a single ovum found in its testes, there were no treatment-related changes in any OPP exposed fish either histopathologically or with respect to gonadal stage. In the absence of other effects, this intersex male can likely be attributed to a non-treatment related

spontaneous change. Thus, because there were no significant effects on assay endpoints observed at any concentration of OPP, the results from this assay indicate that OPP does not have potential for endocrine activity in the hypothalamic-pituitary-gonadal (HPG) axis of fish.

**526 Perfluorooctanesulfonate (PFOS) Exposure Alters Reproduction and Endocrine Function in Fathead Minnows (*Pimephales promelas*)** D. Fort, Fort Environmental Laboratories, Fort Environmental Laboratories, Inc.; J. Autry, Fort Environmental Laboratories, Inc.; M. Mathis, C. Fort, H. Fort, L. Meysing, B. Todhunter, Fort Environmental Laboratories; P. Guiney, J. Weeks, SC Johnson & Son. Exposure of *Silurana tropicalis* to perfluorooctanesulfonate (PFOS) during development was previously found to increase the proportion of phenotypic males, alter steroidogenesis during sexual differentiation, and induce abnormal ovary development. To determine if DE-71 altered reproduction and endocrine function in fish, the Tier 1 EDSP 21-day Fish Reproduction Screen with fathead minnow (*Pimephales promelas*) was used. Reproductively-active male and female fish were exposed to DE -71 ( $< 0.03$ , 0.05, 0.12, 0.29, 0.62, and 1.1 mg/L) via flow-through exposure for 21-d and evaluated for survival, reproductive behaviour, and secondary sexual characteristics. Reproductive fecundity and fertilization success were monitored daily. At termination, the status of the reproductive endocrine system was assessed by the gonad-somatic index (GSI), gonadal histology, plasma steroids (estrogen [E2], testosterone [T], and 11-keto-testosterone), plasma vitellogenin (VTG) and CYP19 (aromatase) activity. Results indicated that the PFOS exposure did not affect survival or body weight, but reduced female GSI. Reproductive fecundity, gonad histology, and endocrinological measures of reproductive status were altered. Increased T was detected in both male and female minnows, and increased 11-KT was detected in male minnows. Decreased plasma levels of E2 and VTG, and CYP19 (aromatase) activity were observed in female minnows. These results demonstrate the anti-estrogenic effects of PFOS on the reproductive system of fathead minnows.

**527 Glyphosate: Amphibian Metamorphosis Assay for the Detection of Thyroid Active Substances** S. Schneider, Wildlife International, LTD / Aquatic Toxicology; H. Krueger, Wildlife International, LTD / Aquatic Toxicology, Wildlife International, LTD; J. Claude, Wildlife International, LTD / Aquatic Toxicology; T. Ross, S. Gallagher, T. Springer, M. Jaber, Wildlife International, Ltd.; J. Task Force, JGTF. An amphibian metamorphosis assay was conducted by Wildlife International, Ltd. for the Joint Glyphosate Task Force at the Wildlife International, Ltd. aquatic toxicology facility in Easton, Maryland from October 24, 2011 to November 14, 2011. African clawed frog (*Xenopus laevis*) tadpoles were exposed to glyphosate at mean measured concentrations of 0.13, 0.79, 4.3, 20 and 90 mg a.e./L for 21 days under flow through conditions. Endpoints that were evaluated to determine if the test substance impacted the HPT axis included survival, gross morphological abnormalities, developmental stage, wet weight, body length, snout-to-vent length, normalized hind-limb length, and thyroid gland histology. There were no treatment-related effects on survival, stage, or normalized hind limb length during the 21-day test. Histopathologic analysis showed no treatment-related changes in the thyroid glands of *Xenopus laevis* tadpoles when compared to negative control animals. There was a slight increase in wet weight in the 90 mg a.e./L treatment group and in snout-to-vent length in the 4.3 and 90 mg a.e./L treatment groups at the end of the 21-day test. However, since there were no effects observed on normalized hind-limb length, stage, or thyroid histology, these increases are not indicative of a thyroid effect. Glyphosate was not found to interfere with the normal function of the hypothalamus.

**528 Glyphosate: Fish Short-Term Reproduction Assay (FSTRA) with the Fathead Minnow (*Pimephales promelas*)** S. Schneider, Wildlife International, LTD; H. Krueger, Wildlife International, LTD / Aquatic Toxicology, Wildlife International, LTD; J. Claude, T. Ross, S. Gallagher, T. Springer, M. Jaber, Wildlife International, LTD; J. Task Force, JGTF. A fish short-term reproduction assay was conducted by Wildlife International, Ltd. for the Joint Glyphosate Task Force at the Wildlife International, Ltd. aquatic toxicology facility in Easton, Maryland from December 21, 2011 to January 11, 2012. The objective of this assay was to determine if glyphosate might impact the hypothalamus-pituitary-gonadal (HPG) endocrine axis resulting in the disruption of reproduction in fish. Breeding groups of fathead minnows (*Pimephales promelas*) were exposed to glyphosate under flow-through conditions at mean measured concentrations of 0.046, 0.23, 1.2, 6.2 and 33



mg a.e./L for 21 days. Endpoints that were evaluated for endocrine disruption of the reproductive system included fecundity, fertility, secondary sex characteristics (including tubercle and fatpad scores), gonadosomatic index (GSI), histopathology of gonads, as well as plasma vitellogenin. Other endpoints included survival, general observations of health, weight, and length. There were no apparent effects on survival, growth, reproduction, secondary sex characteristics, GSI, VTG or gonad histopathology in male or female fish exposed to glyphosate for 21 days. Based on the endpoints evaluated, glyphosate does not appear to impact the function of the hypothalamus-pituitary-gonadal (HPG) endocrine axis in fathead minnows.

**529 Tier 1 EDSP Assays and Regulatory Safety Studies Provide a Weight of Evidence that Glyphosate is Not an Endocrine Disruptor** S. Levine, Monsanto Company / Regulatory Sciences, Monsanto; D. Saltmiras, E. Webb, Monsanto; C. Holmes, S. Mortensen, BASF; J. Honegger, Monsanto; J. Bailey, Dow. The EPA's Endocrine Disruptor Screening Program (EDSP) includes 11 validated Tier 1 *in vitro* and *in vivo* screening assays that evaluate the potential for a chemical to interact with endocrine pathways. The endocrine screening system was designed to have redundancy between the mammalian and aquatic systems. Glyphosate was in the initial group of compounds that received test orders for screening under the EDSP. The compounds in this initial group were selected based on exposure potential and not on known or suspected interaction with the endocrine system. Results from the *in vitro* assays determined that glyphosate is not (anti)-estrogenic or (anti)-androgenic and does not impact steroidogenesis. These findings are fully consistent with peer-reviewed studies using functionally equivalent *in vitro* assays and predictions from structure activity relationships. Additionally, the *in vitro* results from the Tier 1 assays are consistent with the *in vivo* results from existing regulatory safety studies that provide information of the same nature. These higher Tier regulatory studies can provide definitive evidence of endocrine disruption or lack thereof, and are the most powerful tool for hazard identification. Consistent with the results from several multigeneration rat studies, glyphosate exposure had no impact on estrogenic, androgenic, steroidogenic and thyrogenic endpoints in *in vivo* mammalian assays, including the Uterotrophic, Hershberger, and male and female pubertal assays. Consistent the pubertal assays, the amphibian metamorphosis assay found no impact on thyroid-driven developmental endpoints. Results from the short-term fish reproduction study were consistent with the results of an existing fish full life cycle and showed no impact on all measured endpoints. From the weight of evidence provided by the Tier 1 assays, performed at independent labs, under the EDSP along with the higher Tier regulatory safety studies, it can be concluded with a high level of confidence that glyphosate is not an endocrine disruptor.

**530 Assessment of 4,4'-methylenedianiline (MDA) with regard to potential for endocrine modulation effects** R. West, The Dow Chemical Company / Toxicology and Environmental Research & Consulting; R. Jaeger, Bayer Pharma AG / Global Drug Discovery- Product Stewardship: Industrial Chemicals & Operations; M. Collins, Global Isocyanates Limited / Scientific Office. Methylenedianiline (MDA; CAS No. 101-77-9) is included among the U.S. EPA proposed List 2 of substances to be screened for endocrine disruption potential. Assessing chemical substances for possible effects on the endocrine system has become a high profile activity around the world. Definitions, specific assay systems, and tiered testing are still being developed. Nevertheless, the possibility of endocrine modulating effects is one to be considered using the new knowledge against the existing datasets that were in the past not specifically designed to test such endpoints. This presentation first reviews the existing data from animal toxicity studies with MDA for evidence of possible endocrine modulating (EM) effects. In addition, the American Chemical Society Chemical Abstracts databases were searched to identify substances having structural similarity to MDA, and these substances were queried for available studies of endocrine-related effects. The insights from this direct and indirect evidence are summarized to provide a weight-of-evidence which indicates MDA to be of low concern for potential endocrine modulating effects.

**531 Endocrine Disruptor Testing and Screening Program (EDSP): New Data and Implications for Human Health Risk Assessment** K. Connor, ARCADIS. The U.S. EPA Endocrine Disruptor Testing and Screening Program (EDSP) is proceeding with an aim of screening hundreds of chemicals in commerce. The EDSP employs a two-tiered testing regime, where the results from short-term, or *in vitro* assays will determine the need

for longer-term, whole animal studies, with these results presumably serving the development of new or revised Reference Doses (RfDs) for the purpose of human health risk assessment. This paper undertook an evaluation of how the data generated by the EDSP are likely to feed into human health risk assessment, based on the testing regimen and data that are available for several stereotypical endocrine active compounds (EACs). Despite >20 years of intense study, EAC-type effects are hardly influencing the development of RfDs, with only 2 to 3 of the nearly 400 RfDs posted on IRIS having an hormone-like response as the basis. Our review of the underlying data found that even where an endocrine effect, or an outcome that might have an endocrine effect as the basis, e.g., reproductive toxicity, these effects are often not the most sensitive endpoints. Moreover, the RfDs for 7 stereotypical EACs, namely, bisphenol-A, chlorpyrifos, o,p-DDT, methoxychlor, di(2-ethylhexylphthalate (DEHP), PCBs, and vinclozolin, are derived based on endpoints that bear no relationship to their EAC-like activity. The one example of an EAC-like effect being used to derive an RfD is that for TCDD (dioxin) and this RfD is based on human, not animal data. Many have also questioned the value of the EDSP with respect to human health risk assessment, where the Tier 2 testing comes down to standard two-generation reproductive toxicity studies in rats. Based on our review of the 7 above-named EACs, we corroborate this view, finding that there is doubtful connection between hormone-like activity *in vitro* and reproductive toxicity. In conclusion, our findings support the prediction that the EDSP is unlikely to identify substantial numbers of new EACs, and where new EACs are identified, the regulatory path towards a change in RfDs may not be as expected. However, it can be predicted that some positive findings in Tier 1 tests, whether or not corroborated by Tier 2 testing will prompt additional research and heightened public awareness. The need for human health risk assessment addressing the results of short-term, *in vitro* test results and possibly human data will still exist and still present a challenge to risk assessors.

**532 Overview of the Workshop on Ecological Soil Clean-up Values** R. Wentzel, U.S. EPA, ORD / Contaminated Sites/Resource Conserv, Exponent; A. Fairbrother, Exponent. This paper will present an introduction to a recently held workshop to develop a process for establishing ecological soil clean-up values (EcoSCVs). The goal is to progress from ecological soil screening values (EcoSSLs) to final clean-up values by providing regulators with the methods and processes to incorporate bioavailability, normalize toxicity thresholds, address food-web issues, and incorporate background concentrations. Recent major terrestrial research projects, primarily to address REACH requirements in Europe, have significantly advanced our understanding of the behaviour and toxicity of metals in soils. These projects developed large data sets that are useful for risk assessment of metals in soil environments, and were used by workshop participants as case studies in the development of the ecological standards for soils. The workshop attendees discussed scientific advancements in bioavailability, soil biota and wildlife case studies, soil processes, and food-chain modeling. In addition, one of the workgroups discussed the processes needed to frame the topics to gain regulatory acceptance as a directive or guidance by U.S. EPA or the states.

**533 Improvements in frameworks for developing bioavailability-based soil quality standards for metals and metalloids** M. McLaughlin, CSIRO Land and Water/University of Adelaide; S. McGrath, Rothamsted Research / Soil Science Department; R. Lanno, Ohio State University / Department of Evolution, Ecology, and Organismal Biology; R.C. Santore, HDR|HydroQual, Inc.; B. Duncan, US EPA / Region 10. Internationally, most ecological soil quality standards for metals and metalloids are based on total concentrations in soils and take little account of the large effects of soil properties on metal bioavailability and toxicity. This leads to both over-protective and under-protection guidelines depending on the soil conditions encountered. Significant advances have been made in the last decade in our understanding and ability to predict the bioavailability and toxicity of metals and metalloids in soils, and new approaches to developing soil quality standards are now emerging. A workshop was held in September 2012 that explored these recent scientific advances in developing frameworks to improve ecological soil quality standards, and to examine the development of ecological soil cleanup values (Eco-SCVs). One workgroup examined a range of techniques to incorporate bioavailability and long-term fate issues into Eco-SSVs and the appropriate methods to use at each tier of a risk assessment process. Key issues discussed were the general applicability of soil bioavailability normalization relationships, their limitations and data gaps for key metallic contaminants. The state of the science of mechanistic

modeling approaches (terrestrial biotic ligand model – t-BLM) was compared to empirical approaches and key requirements for implementation of the t-BLM for development of Eco-SSVs outlined.

**534 Existing Substance Regulation and REACH in Europe – catalysts for research and improvements in the risk assessment of metals and metalloids in soils** L. Schoeters, Rio Tinto Minerals, Rio Tinto; F. Van Assche, International Zinc Association; K. Delbeke, European Copper Institute; C. Schlekert, Nickel Producers Environmental Research Association; V. Verougstraete, Eurometaux; S. Carey, International Molybdenum Association. Environmental regulations in Europe like the Existing Substance Regulation (793/93/EEC) and more recently REACH (EC 1907/2006) have been catalysts for extensive research projects to better understand the ecotoxicology of metals and metalloids in the environment, including the terrestrial compartment. Under these regulations, risk assessments have been performed on chemicals, including metals, at continental, regional and local scales to ensure they are produced and used safely. At the outset of the Existing Substance Regulation – the predecessor of REACH – the guidance was largely focused on organic chemicals. Experience showed that specific properties of metals such as their natural occurrence, essentiality, and bioavailability had to be considered, but very little guidance was provided for implementing these concepts. Traditional methods such as the application of assessment factors on the lowest ecotoxicity value and even the more advanced Species Sensitivity Distribution method for data-rich substances resulted usually in threshold values below background concentrations of metals in soils. To account for bioavailability in soil risk assessments, the soil parameters influencing metal ecotoxicity first needed to be identified. Equally important was developing representative monitoring databases on distributions of these soil parameters and background soil metal concentrations. The metals industry supported research projects, which allowed the development of models to predict the ecotoxicity of zinc, copper, nickel, molybdenum, cobalt and vanadium in soils. Furthermore, an extensive European database on background metal soil concentrations and soil properties driving the bioavailability and fate of metals has been developed. These findings have resulted in major improvements in the risk assessment methodology for metals in soils, which have subsequently been incorporated into REACH guidance. An overview will be given in this presentation of the regulatory background and the work undertaken by industry to advance the science on ecotoxicity of metals and metalloids in soils to comply with regulations such as REACH.

**535 Recommendations to Improve Wildlife Toxicity Reference Value (TRV) Selection Processes for Soil Cleanup Values** J. Burris, CDM Smith; A. Fairbrother, Exponent / EcoSciences, Exponent, Inc. / EcoSciences; M.S. Johnson, US Army, Public Health Command / Health Effects Research Program, US Army Institute of Public Health / Health Effects Research Program, Army Institute of Public Health / Health Effects Research Program, US Army Ctr for Health Promotion & Preventive Med. / Health Effects Research Program; D.B. Mayfield, Gradient; S. McMurry, Oklahoma State University / Department of Zoology. An integral component of the development of media-specific ecological values for risk assessment involves the derivation of safe levels of exposure for wildlife. Though the derivation and subsequent application of these values can be used for screening purposes, there is a need to identify the threshold for effects when making remedial decisions. A workshop was held in the fall of 2012 that evaluated existing methods and explored recent science for the development of improving ecological soil levels. The purpose of this meeting included a review to improve methods for setting ecological soil screening levels (Eco-SSLs) and to develop ecological soil cleanup values (Eco-SCVs). This required a focused session on the development and derivation to Toxicity Reference Values (TRVs) for wildlife (e.g., birds, mammals, reptiles, amphibians). Topics that were examined included: methods for toxicological endpoint selection, techniques for dose-response assessment, approaches for cross-species extrapolation, and tools to incorporate environmental factors (e.g., metal bioavailability and chemistry) into a reference value. Based on this review, a process was developed to derive wildlife TRVs, using best available science, for setting regulatory soil cleanup values. This TRV process was assessed through a series of case studies for selected metals (e.g., nickel, lead) but is generally applicable to all metals and organic pollutants. The workshop also included recommendations to regulators for how to incorporate the wildlife TRV selection process into the development of soil cleanup levels.

**536 Recommendations to Improve Wildlife Exposure Estimation for Development of Soil Screening and Cleanup Values** B.J. Adams, Rio Tinto / Product Stewardship; C. Menzie, Exponent, Inc., Exponent / EcoSciences; J. Rauscher, U.S. EPA / Region 6; B. Sample, Ecological Risk, Inc.; C. Schlekert, NiPERA. An integral component of the development of media-specific ecological values for risk assessment involves the derivation of safe levels of exposure for wildlife. Though the derivation and subsequent application of these values can be used for screening purposes, there is a need to identify the threshold for effects when making remedial decisions. A workshop was held in the Fall of 2012 that evaluated existing methods and explored recent science for the development of improving ecological soil levels. The purpose of this meeting included a review to improve methods for setting ecological soil screening levels (Eco-SSLs) and to develop ecological soil cleanup values (Eco-SCVs). This required a focused session on the methods for quantifying contaminant exposure for wildlife (e.g., birds, mammals, reptiles, amphibians). Topics that were examined included: methods and models for estimating contaminant bioaccumulation and trophic transfer; contaminant bioaccessibility; possible influence of biodilution on exposure; approaches for addressing essential metals; spatial integration of exposure; dietary vs body burden-based estimates of exposure; and methodological differences between Europe and North America. A process for estimating wildlife exposure for setting regulatory soil screening and cleanup values, based on best available science, was developed based on this review. Applicability of this exposure estimation process was evaluated through a series of case studies for selected metals (e.g., cadmium, nickel, lead) to develop a tiered approach that provides appropriate refinement for metals based on the degree of biomagnification shown by the metal in question. The tiered approach should be generally applicable to all metals and organic contaminants. Recommendations to regulators for integration of exposure estimation methods into the development of soil screening and cleanup levels were developed.

**537 Development of Tier 2 Approaches for Ecological Soil Clean-up Values (EcoSCVs) Protective of Soil Biota and Plants** R. Checkai, The Ohio State University / School of Environment and Natural Resources, U.S. Army Edgewood Chemical Biological Center / Environmental Toxicology, U.S. Army Edgewood Chemical Biological Center / Environmental Toxicology Branch, U.S. Army Edgewood Chemical Biological Ctr / Dept. of Environmental Toxicology; E. Smolders, Katholieke Universiteit Leuven. Significant improvements in test methodology and the development of the new approaches to setting threshold values have gained acceptance in the past 10 years, since the development of Ecological Soil Screening Levels (Eco-SSLs) by the U.S. Environmental Protection Agency. These improvements have provided a basis for further efforts on soil criteria, including a Workshop on Ecological Soil Levels—Next Steps in the Development of Metal Clean-up Values. This workshop, which draws upon experts from Government, Industry, Consultants, and Academia, is organized to provide responsible parties and regulators with methods and processes for moving from screening values to utilization of Tier 2 approaches for establishing soil clean-up values. Development of Ecological Soil Clean-up Values (EcoSCVs) will build upon the Eco-SSL methods and data sets, and incorporate new knowledge and methodologies for soil toxicity testing, addressing and testing for bioavailability, background concentrations, and tools for normalizing toxicity thresholds. In continuity with the existing Eco-SSL methodology that focuses on three receptor groups (terrestrial plants, soil invertebrates, and wildlife), the EcoSCV approach retains a focus on Soil Biota and Plants (Workgroup 2; WG-2). WG-2 will review metals case studies involving plants and soil biota, with side-by-side comparison of Eco-SSL and REACH frameworks. Case studies selected will involve the metals zinc, copper, nickel, and the metalloid molybdenum. The goal of WG-2 will be achieving consensus for Tier 2 soil approaches, evaluating whether methods are appropriate for use in the U.S. decision making; recommendations will be developed on how regulatory agencies should implement use of the proposed methods for EcoSCVs.

**538 Development of Ecological Soil Clean-up Values (EcoSCVs) Protective of Biologically-Mediated Soil Process** R.G. Kuperman, U.S. Army Edgewood Chemical Biological Center / Environmental Toxicology; S.D. Siciliano, University of Saskatchewan / Department of Soil Science; J. Roembke, ECT Oekotoxikologie GmbH; K. Oorts, ARCHE. Significant improvements in test methodology and the development of the new approaches to setting threshold values have gained acceptance in the past 10

years, since the development of Ecological Soil Screening Levels (EcoSSLs) by the U.S. Environmental Protection Agency. These improvements have provided a basis for further efforts on soil criteria, including a Workshop on Ecological Soil Levels—Next Steps in the Development of Metal Clean-up Values. This workshop is organized to provide responsible parties and regulators with methods and processes to move from screening values to clean-up values that address food-web issues, and incorporate bioavailability, background concentrations, and tools for normalized toxicity thresholds. Development of Ecological Soil Clean-up Values (EcoSCVs) will build on the EcoSSL methods and data sets, and will incorporate new knowledge and methodologies for soil toxicity testing. In addition to the three receptor groups used in the EcoSSL methodology (i.e., terrestrial plants, soil invertebrates, and wildlife), the EcoSCV approach includes soil processes that are addressed by Workgroup 5 (WG-5). The main objective of WG-5 is to review the most widely used national and international methods related to the assessment of metal contamination effects on soil processes, and evaluate whether these methods are appropriate for use in the U.S. decision making. The WG-5 will review case studies, the current practices, endpoints, and available data for Zn, Cu, Ni and Mo, and will provide recommendations for regulatory agencies on implementing utilization of the proposed methods for developing EcoSCVs for these metals.

**539 Regulatory considerations for the potential development and application of metal clean-up values** M.S. Greenberg, U.S. EPA Environmental Response Team; I. Schoeters, Rio Tinto; R.S. Wentsel, Exponent; R.G. Stahl, DuPont / Corporate Remediation Group; D.W. Charters, U.S. EPA. A workshop was held in September 2012 to advance the use of soil contaminant bioavailability data, soil parameter data, concentration response relationships, and other newer methodologies in the determination of ecologically protective soil clean-up values (EcoSCVs) for metals that moves us beyond sole reliance on soil screening values as a basis for decisions. This presentation will report on the outcome of the workgroup focused on regulatory applications of the approaches available for developing scientifically defensible EcoSCVs. The U.S. EPA and EU-REACH's, intent and policies and methodologies on ecological risk assessment, derivation of soil screening levels (EcoSSLs), and baseline soil assessments will be discussed with regard to their similarities and differences in how each can lead to site-specific or national management decisions. The utility of Tier 2 processes, common to the REACH guidance for the derivation of soil predicted no effect concentration (PNEC) values and Australian methodology for determining ecological investigation levels on contaminated soils, will be explored through case study examples and discussion of how such a process could be used within the U.S. EPA Superfund process and other regulatory programs to derive EcoSCVs. Additionally, the pros and cons for regulators and the regulated community from the inclusion of such Tier 2 processes for determining EcoSCVs will be discussed. Recommendations from the workgroup on next steps toward additional technical guidance to inform policy-makers will be shared.

**540 Consequential Life Cycle Analysis of Green vs. Grey Infrastructure for Stormwater Management** R. Wang, Yale University; M. Eckelman, Northeastern University; J. Zimmerman, Yale University. A life cycle analysis of four green to grey infrastructure technologies for stormwater management was performed as compared to a baseline of the current situation in many urban areas, combined sewer overflows. The four interventions include sewer separation, porous pavement, bioretention, and green roofs. Four impact categories were considered (greenhouse gas emissions, toxicity, eutrophication, and resource depletion) under four precipitation scenarios that increase in quantity and intensity to mimic predicted impacts of climate change on storm events. Life cycle credits were also considered for offsetting the traditional technology (i.e., asphalt pavement versus porous pavement). A sensitivity analysis was performed and demonstrated that infrastructure lifetime and storm event quantity and intensity were the most significant drivers for the life cycle implications of these systems.

**541 Development and Use of Fundamental Service Life Models for LCA of Advanced Infrastructure Materials** M.D. Lepech, Stanford University / Department of Civil and Environmental Engineering. Concrete transportation infrastructure lies at the nexus of two significant sustainability challenges; global greenhouse gas emissions associated with cement production and vehicle energy consumption and toxic tailpipe emissions. The use of advanced engineering materials in infrastructure can significantly reduce

these impacts through reduced material consumption, improved durability leading to reduced congestion traffic emissions, and enhanced end-of-life management. Engineered Cementitious Composites, or ECC, are a new cement-based infrastructure material that exhibits ductility over 300 times that of conventional concrete. Such ductility can lead to significant reductions in material use and vehicle impacts through extended maintenance and replacement timelines. Previous studies have demonstrated the potential environmental impact reductions associated with this increase in life cycle performance. While Life Cycle Assessment (LCA) is a powerful tool for quantifying the sustainability benefits of advanced materials in highly durable applications such as infrastructure, it remains unconvincing due to the high uncertainty of the future performance of unproven materials. Recent work in service life modeling of reinforced concrete and ECC materials is addressing this shortcoming. Based on fundamental, multi-phase porous media transport and electrochemistry modeling, new service life models for both existing and new infrastructure materials are presented. These models capture the time-dependent deterioration of reinforced concrete infrastructure materials from initial transport of corrosive species, to electrochemical depassivation of reinforcing steel, to active corrosion, and up to end of service life and replacement. Integrating such fundamental deterioration and service life models into life cycle assessment of infrastructure can more clearly demonstrate the long term benefits of advanced materials over existing infrastructure construction. Such models enable probabilistic characterization of expected service life of new materials, such as ECC, thus allowing for more rational consideration of future uncertainties. More generally, the benefits of integrating fundamental service life modeling with LCA is discussed in the context of unproven, yet highly promising advanced materials for a variety of applications.

**542 The misunderstood role of construction technologies in the GHG mitigation of the building stock** J. Heinen, Aalto University / Department of Planning and Surveying; S. Junnila, Aalto University. Building energy use is accountable for approximately 40% of the global greenhouse gas (GHG) emissions. In Finland housing energy is by far the most dominant sector in the carbon footprint of an average consumer. Construction phase emissions, on the other hand, are generally held unimportant compared to the use phase emissions. Based on this, often drawn policy implication is that increasing the building energy efficiency would be a very effective GHG mitigation strategy. Accordingly, the efficiency requirements on residential heating energy consumption in Finland have tightened from 185 kWh/sq. m in 1985 to 80 kWh/sq. m in 2012. As a consequence, construction technologies have taken long steps forward to meet these new requirements. While this development gradually reduces the overall energy consumption of buildings as the building stock renews, little attention has been paid to the construction phase emissions in the building life cycle due to the prevailing belief about their insignificant role. However, while construction related emissions of a building may seem small on national level accounts as well as compared to the overall use phase emissions, the situation changes significantly when a certain development project is concerned and the temporal allocation of the emissions is taken into account. With the current rather high energy efficiency requirements in Finland, the construction phase emissions may actually dominate the building related emissions for decades. Furthermore, when the construction phase emissions are taken into account, despite the highly increased efficiency requirements, the current approximately 1% annual renewal rate of the building stock in Finland only leads to an increase in the cumulative emissions up to 2050, the current target year of significant mitigation needs. Thus, it seems that partial optimization of the life cycle emissions without understanding the broader context might lead to weak policy guidelines and unwanted consequences. In this study we demonstrate the above described phenomena with a hybrid life cycle assessment (LCA) model that is able to take into account the temporal allocation of the emissions. We also analyze solutions to decrease the construction phase carbon spike concentrating especially on wood construction technologies. We employ a case study of a new residential development from Finland and broaden the perspective with a bottom-up approach to the national level for the overall conclusions.

**543 LCA Study of Artificial Photosynthesis—hydrogen compression/liquefaction using photovoltaics** P. Zhai, H. Breunig, R. Sathre, J. Greenblatt, Lawrence Berkeley National Laboratory. Artificial photosynthesis (AP), also known as photo-electrochemical devices for solar water splitting, harvests sunlight and converts it to energy stored in hydrogen bonds.



Although AP is still in the early stage of laboratory scale research, AP could change the future energy landscape. LCA and energy balance analyses are essential for evaluating the potential of this emerging technology. We investigate the prospective energy balance of an AP system. The LCA boundary of the system includes embodied energy in raw materials and manufacturing processes of AP panels; and energy requirements for hydrogen compression/liquefaction. The latter issue is the focus of this study. Previous studies have reported significant variation of energy requirements to compress or liquefy hydrogen due to differences in assumed final pressure (500 or 1000 psi), modes of transportation (trucks or pipelines), and boundary definitions (inclusion of hydrogen dispensing). We propose several scenarios of energy requirements based on a comprehensive literature review. Our study is unique in that renewable energy will be implemented to minimize the primary energy required to power the compression or liquefaction of hydrogen. We predict that photovoltaics (PV) and AP could work synchronously during day time. We will examine how PV meets the electricity demand to handle hydrogen generated by AP, and assess related embodied energy, cost and land use issues caused by PV installation. Finally, we will compare the life cycle energy balance of an AP system under two cases: first, powering hydrogen compression and liquefaction using PV electricity; second, using electricity from a power grid (assuming U.S. average power mix).

**544 Developing an Electric Vehicle Impact Assessment that Incorporates Uncertainty from the Deployment of Life-cycle Processes** M. Chester, Arizona State University / Civil, Environmental, and Sustainability Engineering. Advancements in transportation environmental life-cycle assessment should incorporate spatial dimensions and more rigorous human health and environmental impact assessment for emerging systems. Using electric vehicles as an example, this presentation will discuss new approaches that have been developed to evaluate the uncertainty in spatial characteristics of future life-cycle inventories and the human health and environmental damage costs of emissions. The electrification of passenger vehicles has the potential to produce fewer greenhouse gas emissions than gasoline, reduce and displace tailpipe emissions, and reduce dependency on imported oil. Federal policy has been created to encourage development and deployment by subsidizing vehicles up to \$7500. Yet the benefits of emerging electric vehicles have been studied with somewhat myopic lenses. Many studies have been developed to evaluate the energy and GHG tradeoffs of electric vehicles over gasoline vehicles, and few have considered human health and environmental tradeoffs. Only a handful of studies to date have attempted to comprehensively quantify benefits and costs. By joining damage cost estimates with an uncertainty analysis of emission release locations, the greenhouse gas, human health, and environmental impact costs of conventional gasoline, plug-in hybrids, and battery electric vehicles are determined for driving in 3,000 U.S. counties. In the short run, vehicles with small battery packs (i.e., HEVs and low-range PHEVs) produce the lowest life-cycle costs and policies that provide larger subsidies to large battery pack vehicles do not generally offer more benefits. For the average U.S. electricity grid mix, a conventional gasoline vehicle produce \$4800 damages in greenhouse gas, human health, and environmental effects, HEV \$3900, PHEV20 \$3600, PHEV60 \$4100, and BEV \$4700. These costs are largely dominated by emissions of greenhouse gases and sulfur dioxide. Combining these costs with oil displacement effects provides a comprehensive and unifying framework for evaluating emerging vehicle impacts. Furthermore, the spatial characterization of effects presents opportunities for avoiding the deployment of direct, indirect, and supply chain processes to high-impact areas.

**545 The role of biofuels in the electrification of US passenger transportation** C.D Scown, Lawrence Berkeley National Laboratory; A. Horvath, University of California, Berkeley / Civil and Environmental Engineering, University of California, Berkeley / Civil & Environmental Eng.; T. McKone, University of California, Berkeley, University of California and Lawrence Berkeley National Laboratory, University of California, University of California and Lawrence Berkeley National Laboratory, University of California. A variety of alternative transportation fuels have been explored over past decades, but most are difficult to produce at the scale of current gasoline demand on the timeframe necessary to meet national greenhouse gas (GHG) emissions reduction goals. Rather than exploring each option individually, potential fuels should be analyzed in combination through long-term scenarios that take advantage of different fuel pathways' strengths while ensuring a smooth transition and a sustainable end state. Electrifying passenger transportation is at present one of the most promising options for

achieving lower carbon emissions, with greater long-term benefits if the US is able to shift from coal and natural gas-fired power plants to renewables such as wind and solar. However, most electric vehicles (EVs) and plug-hybrid electric vehicles (PHEVs) have limited ranges and long recharging times. Their fleet penetration rate is also limited, as these vehicles are more costly than their traditional gasoline-powered counterparts. Cellulosic ethanol, while unable to displace all US gasoline demand without significant land use change impacts, has some advantages over electricity: its storage and distribution can be integrated with existing liquid fuel storage and distribution infrastructure, and flex-fuel vehicle technology that allows for burning of 85% ethanol by volume is relatively inexpensive and already present in many vehicles on the road. Additionally, preliminary results show that low-input high-yield crops such as *Miscanthus* can ultimately result in ethanol with a net negative GHG footprint, with higher-emission scenarios still averaging only 22 g CO<sub>2</sub>e/MJ of ethanol. US county-level scenario analysis extending to 2050 is used to explore the possibility of utilizing crop residues and dedicated cellulosic crops to meet liquid fuel demand as PHEVs gradually penetrate the passenger vehicle fleet. Life-cycle assessment is used to determine possible emissions reductions under conservative, moderate, and aggressive scenarios. Results show that cellulosic ethanol has significant potential to deliver necessary short-term GHG emissions reductions as PHEVs and EVs achieve market penetration, and may also satisfy residual liquid fuel demand in the long term.

**546 Energy Production and Nutrient Recovery from Residual Algal Biomass** W.J Barr, A. Landis, Arizona State University / School of Sustainable Engineering and The Built Environment. Algae biodiesel has been shown to be a promising alternative fuel source. However, the production of biodiesel from microalgae is an energy intensive process and despite being produced from sunlight, still requires fertilizers and other chemical inputs. A significant portion of waste algal biomass remains unused after the lipid extraction process. This lipid-extracted algae (LEA) contains the bulk of the nutrients consumed during cultivation and has the potential to be used for a secondary energy source via methane production. Using a life cycle assessment (LCA) framework, this presentation tracks energy and nutrient production and consumption over the life cycle of algae produced in a photobioreactor. Anaerobic digestion (AD) is used to recover nutrients and energy from LEA. The feasibility of using AD to recycle nutrients and energy within the biodiesel production facility will be assessed through a computational LCA. Alternative technologies will be evaluated to improve AD yields. In addition, the life-cycle implications of improving on the energy recovery and nutrient recycle within the algae biorefinery will be discussed. AD has the potential to improve the net energy balance of algae-based biodiesel production by recycling energy and nutrients inside of the system that would otherwise be wasted.

**547 Baseline effects on carbon footprints of biofuels: the case of wood** E. Johnson, Atlantic Consulting. As biofuel usage has boomed over the past decade, so has research and regulatory interest in its carbon accounting. This paper examines one aspect of that carbon accounting: definition of the baseline, i.e. the reference against which other conditions or changes can be compared. A literature search and analysis identified four baseline types: no baseline; reference point; marginal fossil fuel; and biomass opportunity cost. The fourth one, biomass opportunity cost, is defined in more detail, because this is not done elsewhere in the literature. The four baselines are then applied to the carbon footprint of a wood-fired power plant; all other inputs and calculations are identical. It is found that the footprint of the resulting wood-fired electricity varies dramatically, according to the type of baseline. Baseline type is also found to be the footprint's most significant sensitivity. Other significant sensitivities are: efficiency of the power plant; the growth (or re-growth) rate of the forest that supplies the wood; and the residue fraction of the wood. Length of the policy horizon is also an important factor in determining the footprint. The conclusion is that because of their significance, baseline choices should be made very explicit in biofuel carbon footprints. The 'no baseline' approach is not acceptable. The other three approaches are acceptable, but their suitability to a particular generation plant (of power, heat or both) depends on the type of question to be answered.

**547.1 Introduction to the Proposed Safer Consumer Products Regulations** E. Rodriguez, Department of Toxic Substances Control / Pollution Prevention and Green Technology. California's Green Chemistry law (A.B. 1879) was adopted to accelerate the quest for safer product. The enabling

statue requires the Department of Toxic Substances Control (DTSC) to adopt regulations that reduce or eliminate adverse public health and environmental impacts that may result from the production, use, or end-of-life management of consumer products containing chemicals of concern. This presentation will cover the major steps being proposed to provide a continuous, science-based, iterative process to identify safer consumer product alternatives. First, the proposed regulation will specify the processes for DTSC to identify and prioritize chemicals of concern in consumer products. The initial list of chemicals of concern will be established based on the work already done by other authoritative organizations. DTSC will evaluate and prioritize product/chemical of concern combinations to develop a list of "Priority Products" for which an alternatives assessment must be conducted. The next step in the process requires the manufacturers to conduct an alternatives analysis which compares health, environmental and economic trade-offs of those product/chemical combinations with potentially safer alternatives to replace or eliminate prioritized chemicals of concern in products. Alternative analysis will be required to cover a wide range of factors over the lifecycle of the product. Lastly, based on the findings of the alternatives analysis, DTSC may impose regulatory responses that will effectively limit potential adverse public health and/or environmental impacts, if any.

**548 Multi-State Effort to Create an Alternatives Assessment Guidance** A. Stone, Wa Department Of Ecology / Chemist. In 2008, the Washington Department of Ecology (Ecology) was required by the Washington Legislature to conduct an assessment of alternatives to the flame retardant, decabromodiphenyl ether. If a safer alternative could be identified, a ban would take effect on the use of deca-BDE in specific applications. Ecology used the GreenScreen methodology created by Clean Production Action and based upon the principles developed by EPA's Design for the Environment Program. The Deca-BDE ban took effect in 2011 and Ecology has selected the GreenScreen as the hazard assessment tool it will use in all future alternatives assessments. Subsequently, eight member states of the Interstates Chemical Clearinghouse began working together in August 2011 to create an alternatives assessment (AA) guidance document. The AA guidance consists of twelve modules with each module representing an important component of an AA. Modules include such basic concerns as cost and availability, performance, hazard, exposure, stakeholder involvement etc. and includes more complex issues as life-cycle analysis. Each module consists of a number of levels of increasing complexity and assists the user in identifying what information is needed to contribute to an AA for the specific issue being addressed. During this process, the states have sought out input from a wide range of stakeholders and has worked with business to flesh out a guidance document that can be used by users with varying levels of technical expertise and experience. One important objective of the guidance is to create a document that can be used by small and medium businesses as well as the larger concerns that are accustomed to conducting similar assessments. It will also prove useful to state, local and national governments who may be evaluating AAs in the future. The current schedule has the completion of this guidance document by the end of 2012. The objective of this talk is to provide an update on the process, a description of the guidance and a summary of the experience of the states in working together to create the guidance. In addition, a description will be provided on how Washington State sees the document will assist us in the Agency goal of Reducing Toxic Threats by eliminating or reducing the use of toxic chemicals in consumer products and better protecting human health and the environment.

**549 Substitution and Alternative Assessment Tools Under State Green Chemistry Programs: Learning from the International Experience** D. Graham, Keller & Heckman LLP; D.J. Kent, H. Estreicher, Keller and Heckman LLP. The California Green Chemistry regulation has as its cornerstone the systematic substitution of Chemicals of Concern in consumer products with "safer" chemicals. Washington State leads the development of the Alternatives Assessment (AA) guidance for the Technical Alternatives Assessment Guidance Team (TAAG) which is made up of representatives from the seven states who are members of the Interstate Chemicals Clearinghouse. The idea of substituting chemicals is not new and has long been a core principle in Europe as part of REACH Authorization, which requires the ultimate phase-out of substances of very high concern. Several years ago Canada began its process of identifying high hazard chemicals which it has been listing as CEPA Toxic. As with the EU and Canadian programs, the proposed AA guidance is primarily based on hazard assessments, but does include an exposure module. Recent experience with chemical substitution

including with some products advertised as "Green" indicates that substitution may result in different kinds of exposures and possibly adverse health outcomes. This presentation looks at how California and other states can build on the experience of other jurisdictions in structuring programs that will result in the desired outcome of reducing human and environmental exposure to hazardous chemicals.

**550 Case Study Application on Green Screen for Safer Chemicals to the U.S. Chemical Manufacturing Industry** C.W. Lam, University of California, Davis / Department of Chemical Engineering and Materials Science; C.W. Lam, J.M. Schoenung, University of California, Davis / Chemical Engineering and Materials Science. Heightened societal interest to reduce toxicity potential from chemical products and processes has spurred governmental agencies, company programs and public consumers to procure and use chemicals which are inherently safer. As a milestone effort in defining "safer", the Clean Production Action (CPA) developed Green Screen for Safer Chemicals as a hazard screening tool that considers a comprehensive range of human health, ecological, and safety hazard traits of substances so that benchmark scores, an indication of relative hazard, could be assigned for specific chemicals. Although Green Screen has recently seen increased attention within select regulatory and industrial circles, outside of a limited number of individual chemical case examples, no independent, academic-based published work is available to test Green Screen for a larger range of chemicals. Such an exercise is important to understand, from an aggregate perspective, the potential challenges it has in application for diverse substances. The goal of this research consists of using Green Screen to analyze a select list of chemicals to survey the tool's use and, more importantly, to discuss its attributes and limitations. The evaluation covers over 35 chemicals most commonly emitted to air and water media from the United States' chemical manufacturing industry as reported by the Toxic Release Inventory (TRI). Preliminary results will be presented on benchmark scores of these TRI chemicals using publicly available toxicity information. Toxicity data gaps are highlighted as an issue for developing complete Green Screen assessments, even for regulated TRI chemicals which are common and released in high quantities in the U.S. Despite data gap concerns, benchmark scores are able to be assigned for TRI chemicals, with the majority of substances falling into benchmark 1 (avoid – chemical of high concern) and 2 (use – but search for safer substitute) categories.

**551 Application of LCA in safer products alternatives analysis- a California perspective** B. Boughton, CalEPA / Calif. Dept of Toxic Substances Control, DTSC. Life cycle thinking is an essential component of alternative assessment (AA). As applied to product design and production processes it informs decision-making. The goal of Life Cycle Assessment is to make sure that reducing an impact at one stage in the life cycle does not increase an impact at other parts in the life cycle. Life cycle thinking should be employed in alternatives analysis because it leads to informed substitution and allows one to avoid unintended consequences. Businesses are responsible for many choices about their services and products. Businesses should not only take into consideration how the product is made, but also ways that the product will be used and disposed of by the user. When considering alternatives, they should study how each design and manufacturing decision has a net effect on the environment and human health. By looking at all of the life-cycle phases, businesses can make the best choices for a lower net impact on the environment and society. The current alternative analysis guidance approach to comply with the California Safer Products Regulations (see [www.dtsc.ca.gov/PollutionPrevention/GreenChemistryInitiative/index.cfm](http://www.dtsc.ca.gov/PollutionPrevention/GreenChemistryInitiative/index.cfm)) will be presented. This is a decision framework for evaluating alternatives for products containing chemicals of concern to human health and the environment. Application of life-cycle thinking in alternatives analysis can be done at several levels. The levels described build from a qualitative cursory review of all attributes (using screening tools) to quantitative approaches for the most relevant aspects. With this approach, effort is focused on the attributes that most matter, rather than conducting a full ISO compliant LCA.

**552 Safer surfactants – How to develop chemicals with lower antimicrobial toxicity** N. Gathergood, Dublin City University / School of Chemical Sciences; M. Spulak, Charles University / Department of Inorganic and Organic Chemistry, Faculty of Pharmacy; B. Quilty, Dublin City University / School of Biotechnology; D. Coleman, Dublin City University / School of Chemical Sciences. One of the reasons that the chemical industry has great interest in Green Chemistry is the financial benefits. By developing greener

and cleaner methods and processes, that inherently generate less waste, the costs associated with waste disposal can be reduced. Industry can reduce their environmental impact as well as increase profitability. In their book, Anastas and Warner proposed the 'Twelve Principles of Green Chemistry' primarily to inspire chemists to create environmentally less damaging synthetic methodologies and products. Our own research team in Dublin encompasses a variety of these green chemistry principles to realise environmentally friendly organic synthesis for chemicals of interest to industry. Concurrent assessment of the performance of a new chemical with its potential environmental impact enables our group to focus investigations on 'safer' novel chemicals. The development of reactions with high atom economy, to reduce waste products, combined with low toxicity and biodegradable reaction media, is one example of our benign-by-design philosophy. Recent examples from our search for low toxicity and biodegradable surfactants will be presented. In addition our novel approach for the design of low toxicity surfactants also yielded a MRSA selective antimicrobial drug which is highlighted in Chemistry World and in national press (The Sunday Times).

**553 Experiences with Alternatives Analysis and Product Development in Formulated Products** D. Versteeg, The Procter & Gamble Company / Environmental Science Department; M. Lafronconi, J. Froelicher, B. Percynski, The Procter & Gamble Company. California's Green Chemistry Initiative and Safer Consumer Products regulations seek to enhance the safety of consumer products for humans and the environment by applying an alternatives assessment (AA) process to identify replacements for ingredients of concern. In doing so, the law goes beyond exposure and toxicity concerns related to individual ingredients by including factors such as energy inputs, energy consumption, water conservation, material consumption, etc. The AA is the primary tool used to identify new ingredients and other technical solutions presented by the ingredient of concern. The AA process in California, and the ones evolving in other states, are similar to research and development approaches industry has applied for decades to create new and improved products. The product development process is iterative and necessarily flexible to account for product and consumer specific needs. To be successful, the process must not only consider use and toxicity, but also supply, aesthetics, compatibility, stability, and a range of other manufacturability issues. Experiences with successful and unsuccessful AAs will be shared.

**554 Applying the GreenScreen for Safer Chemicals to Electronics Cleaners** C. Wray, C. Robertson, H. Holder, P. Mazurkiewicz, Hewlett-Packard Company. Hewlett-Packard has been incorporating hazard assessment requirements into its global materials selection process in order to reduce the environmental and human health impact of its products and manufacturing processes. HP utilizes a comparative Chemical Hazard Assessment tool called the GreenScreen for Safer Chemicals (GreenScreen) to ensure that the alternative materials are indeed less hazardous than those being replaced. Based on the principles of green chemistry that focuses on reducing risk by reducing hazard, the Green Screen provides a simple 1-4 benchmark score that can help identify less hazardous chemical and non-chemical alternatives. A business case for screening alternatives using the Green Screen can be made based on the cost savings realized by avoiding multiple substitutions. Substituting materials in a complex supply chain can be costly, and if the replacement chemicals face future regulation, businesses may incur that cost multiple times. This presentation will introduce the GreenScreen as well as discuss its application to the hazard assessment of cleaners used in electronics manufacturing.

**555 A model based adaptive control of pest in agriculture: An application on cotton crop** M. Yanke Nana, Technische Universität Braunschweig / Environmental Systems Analysis, Institute of Research in Health Sciences; O. Richter, Technische Universität Braunschweig / Environmental Systems Analysis. Agricultural pests control is crucial for a better crop yield. Until now, the best method of struggling against pests in agriculture is the use of crop protection products, which can cause huge damages to the environment. Therefore, there is a need for an optimal crop treatments schedule. As such, efforts have been made towards the development of simulation models to forecast pest's population size linked to its response to a dose of a specified pesticide, including the potential contamination of the environment. The simulation model was developed for cotton crop influenced by the pest *helicoverpa armigera* (HA). This model will lead to a more appropriate and adaptive pest control using pesticides in agriculture, with respect to the environment. An integrated concept comprising submodels for cotton growth,

population dynamic of HA and its application to the evaluation of optimal treatment scheme will be presented.

**556 A step towards spatially realistic simulations of pesticide effects on aquatic macroinvertebrate populations** A. Focks, Environmental Systems Research / Mathematics/Computer Science, Wageningen University / Aquatic Ecology and Water Quality Management, Wageningen UR / Aquatic Ecology and Water Quality Management; N. Galic, Wageningen University; H. Baveco, Alterra Wageningen; P.J. van den Brink, Wageningen University. Little is known about ecological risks of chemicals considering spatial heterogeneity on larger scales, especially with regards to the population recovery potential after exposure to chemical stress. This study aims to evaluate the influence of i) persistence and toxicity of hypothetical pesticides and ii) different levels of spatial complexity on effects and recovery times of populations of the water louse *Asellus aquaticus*. To reach these objectives, the MASTEP population model of *Asellus aquaticus* (Galic et al., 2012) was used in a spatially realistic structure of a typical Dutch water ditch network. Spatio-temporal concentration curves were calculated for a set of virtual pesticides. Exposure concentrations were linked with effects on individual organisms by calculating daily mortality probabilities following a simple dose-response relation. Scenarios were simulated in 10 replicates each, and the recovery times have been calculated as the number of days, when population abundances in treated scenarios reached 95% of the level of the abundances in respective control simulations. Our analysis showed the importance of chemical properties in a spatially explicit setting. Both persistence and toxicity of the simulated compounds influenced the magnitude of effects on and subsequent recovery times of the water louse populations. At high toxicities, increasing water network complexities lead to shorter recovery times of the affected populations. This is quite well understood, because recovery can occur via i) recolonisation from unaffected habitats and ii) local population regrowth enhanced by survivors. High toxicities lead to "wipe-out" events in the downstream section, so local regrowth only plays a minor role in the recovery. In this case the connectivity of the affected sections of the water network to unaffected parts is decisive for recovery. The lower the toxicity, the more local regrowth contributes to recovery and hence the spatial structure is less important. Implications of our research are: i) the use of existing population effect models in simplified landscapes are protective within the limitations of any modelling approach, and ii) assessing the recovery potential and quantifying the time to population recovery is very sensitive with respect to changes in pesticide and landscape properties, so integrating the detailed exposure profiles through e.g. pesticide fate model output, will inevitably lead to a more realistic ecological risk assessment.

**557 Adding ecological relevance to risk assessment: modeling the effect of multiple and heterogeneous stress on springtail populations** M. Meli, Roskilde University / Department of Environmental, Social and Spatial Change; A. Palmqvist, Roskilde University / Dept. of Environmental, Social & Spatial Change; V.E. Forbes, University of Nebraska Lincoln / School of Biological Sciences; V. Grimm, Helmholtz Centre for Environmental Research – UFZ / Department of Ecological Modeling. In assessing soil quality and the environmental and ecological risk of contamination with heavy metals, often homogeneous contamination is assumed. However, soils are very heterogeneous environments. Consequently, both contamination and the response of soil organisms can be assumed to be heterogeneous. Moreover, in the natural environment organisms are likely to be confronted with physical stressors, e.g., summer drought. To explore the consequences for populations of soil invertebrates caused by the interaction of chemical and physical stressors, we developed a spatially explicit individual-based model of the collembolan *Folsomia candida*. Individuals in the model can sense and avoid contaminated habitat with a certain probability, which depends on contamination level. Avoidance of toxicant influences the feeding behavior of the organisms, and this in turn affects all the other biological processes. Model rules and parameters are based on previous knowledge of the biology and ecology of the species; for toxicity, data from standard laboratory tests (survival, reproduction and avoidance) are used. Heterogeneity of soil contamination with  $\text{CuSO}_4$  was varied according to the percentage of contaminated area and the spatial autocorrelation of the contaminated patches of soil. In addition, we simulated the effects of drought on collembolan populations, with and without Cu exposure. Preliminary simulation results show that the presence of patches of suitable habitat allows the population to grow, although population size is reduced with respect to the control. Toxic effects are much less than proportional to the concentration,



because avoidance probability increases with increasing Cu concentration. When the uncontaminated area is small (< 5%), stable population size is bigger in the case of spatially correlated distribution of toxicant, whereas as the proportion of clean habitat increases, population growth is higher with uncorrelated contamination. Our results show that a combination of spatial heterogeneity and physical stressors can lead to unexpected effects of toxicants at the population level. Individual-based models can help to understand these effects and therefore can add ecological realism to environmental risk assessment of chemicals.

**558 Density in population-level ecotoxicology and risk assessment: insights, hypotheses and uncertainties from a density dependent simulation model** C.J. Salice, Texas Tech University / Environmental Toxicology, Texas Tech University / TIEHH, Texas Tech University / Department of Environmental Toxicology.

Population models are a powerful way to integrate and assess the ecological impacts of chemical contaminants. A particular challenge, however, lies in understanding and modeling the interaction of contaminant effects and density dependence. How contaminants and density interact and are modeled can have profound impacts on our interpretation of overall population-level responses. While there has been some research on the topic, considerable uncertainties remain. A common view is that density dependent compensation will limit adverse demographic effects of contaminants. Although there is some empirical evidence for this, it is not unilaterally the case. In an effort to better understand the interactions between density dependence and toxicity, I used a previously published amphibian population model that incorporates a four-parameter density dependent function to explore and define under what circumstances toxicants may cause positive or negative effects on the population. I also used the model to explore the utility of several population-level endpoints including population growth rate, population size and extinction risk. The interactions between toxicity and density dependence were simulated as toxicity: (1) reducing maximum survival, (2) altering the functional availability of resources and (3) changing the function inflection (curve shape). Positive population level effects result when toxicants reduce the initial number of individuals thereby lessening density dependent stress. This, functionally, amounts to an increase in resource availability but assumes there is no lasting impact of toxicity. Alternatively, a range of different toxicant and density interactions can result in negative population level effects, stemming from a reduction in maximum survival, a change in the shape of the density dependent function and/or an alteration in functional availability of resources. Hence, simplistic approaches in which density effects are accounted for after a reduction in individuals may not accurately capture population dynamics. The population endpoint that provided the most insight and management utility was population size followed by population growth rate and extinction risk. These results have strong implications for the protection of wild populations and point to the importance of continued research and model development and are further discussed in terms of a critical can of worms: selecting management endpoints.

**559 Determining chemical effects on earthworms at the population level through the use of energy budget and individual based modelling** A.S. Johnston, R. Sibly, University of Reading / Biological Sciences; M. Hodson, University of Reading; P. Thorbek, Syngenta Ltd / Environmental Safety; T. Alvarez, Syngenta / Dept. of Ecological Sciences, SRG / Syngenta / Product Safety. Standard OECD test methods for measuring the toxicity of pesticides on earthworms use optimal laboratory conditions to record specific endpoints e.g. mortality, growth and reproduction. These data are compared to exposure values in accordance with guidelines for registration of agrochemicals, and the resulting risk quotients are compared to arbitrary safety factors for acute and sublethal risks to earthworms. When these triggers are not passed, field trials are required. Field trials are expensive, time-consuming and variable, providing coarse information about population-level effects. Mechanistic population modelling of organism responses to chemicals has the potential to act as a reliable intermediate stage in higher tier risk assessments and can provide greater resolution to help identify key parameters driving population-level effects. Energy budget models relate individual life cycle processes to one another through energy and mass conservation. Organisms uptake resources from their environment and expend assimilated energy on maintenance, growth and reproduction. The allocation of energy to these different subsystems depends on a combination of environment- and organism-specific conditions. Here we present a general energy budget model, based on broadly accepted fundamental principles.

The model is applied within an individual-based model (IBM) framework, which allows individuals to interact with their simulated environment, giving an insight into life cycle processes affected under variable environments and chemical exposure. This enables ecotoxicological inferences to be made about population dynamics under realistic field conditions, as the biology of individuals relates to the ecology of populations through metabolism. Standard toxicity data, including NOEC and EC<sub>50</sub> values, are used as input data to the model to hypothesise possible modes of action, highlighting its usefulness as a method in higher tier risk assessments. We use data for the OECD recommended earthworm species *Eisenia fetida* to validate the model for use in predicting ecotoxicological effects for a variety of chemicals, including carbendazim and copper oxychloride. We discuss the potential of the model in decision-making for pesticide regulation and environmental management, particularly in comparison to alternative modelling techniques. Future studies aim to extrapolate population-level ecotoxicological effects between the varied ecological niches of different earthworm species.

**560 Extrapolating individual reproductive toxicity to long-term population level endpoints using an individual-based model of the wood mouse** C. Liu, Syngenta / Environmental Safety; R.M. Sibly, University of Reading / School of Biological Sciences; V. Grimm, Helmholtz Centre for Environmental Research – UFZ / Department of Ecological Modeling; R.C. Murfitt, P. Edwards, Syngenta / Environmental Safety; P. Thorbek, Syngenta Ltd / Environmental Safety.

In EU pesticide risk assessment of potential chronic effects upon wild mammals, the “two-generation reproduction toxicity study” is commonly used to select a toxicity endpoint for risk assessment. Reproductive performance as well as maternal behaviour of the test individuals is followed under long-term exposure until the second generation are produced, to allow a thorough examination of the full life cycle (i.e. growth, development and reproduction) in the lab. However, such studies focus on individual level effects whereas the protection goal is at the population level. Population modelling can extrapolate such data from individuals to population level endpoints. Here we present an individual-based model of the wood mouse (*Apodemus sylvaticus*) aimed at refining pesticide risk assessments in agricultural landscapes. The full life cycle of female wood mice and their spatial behaviour, in particular the choice of foraging and nest sites, were explicitly modelled. Additionally, the model implemented a dynamic landscape with spatio-temporal variation of the habitat quality as a result of crop growth, rotation scheme and tillage activities. By linking the spatial choice of the mice and the application patterns of the pesticides, the model provided more realistic estimates of exposure in different habitats at different time of the year. We used the model to explore the population level sensitivity (level of exposure, population structure, time to recovery) to different individual level endpoints. We tested different application schemes (foliar and seed treatment) and found that the ecological relevance of these individual level endpoints varied widely. Based on the predictions from the model, we showed how such model outputs can be used to refine higher tier risk assessments.

**561 A simplified AQUATOX ecosystem model for endocrine disruption** L. Clouzet, Université Laval / modelEAU; M. Paterson, A. Dupuis, P. Blanchfield, P. Blanchfield, Fisheries & Oceans Canada. Endocrine disruption is widely studied at the individual level but the effects on population and ecosystem dynamics remain unclear. Mechanistic models can help understand the impact of endocrine disruptors on aquatic environments and assess their ecological risk. However, the issue with modeling endocrine disruption is to find the appropriate endpoints that represent the reproductive disturbances observed in fish. This study takes the challenge to develop an ecosystem model that considers endocrine disruption in fish and the consequences on the whole ecosystem through ecotoxicological interactions, i.e. feeding and competition relationships. The experimental data used to develop this model come from a multi-year whole-ecosystem study performed at the Experimental Lake Area (Ontario, Canada). The synthetic hormone 17 $\alpha$ -ethinylestradiol (EE2), one of the most potent endocrine disruptors, was added during three years in an experimental lake at environmentally relevant concentrations. Experimental data were collected before, during and after EE2 addition. Endocrine disruption was observed in the fish species with a collapse of fathead minnow after the second year of EE2 addition. The ecosystem model that is being developed is an object-oriented model based on simplified AQUATOX equations for the species naturally present in the experimental lake (benthic invertebrates, phyto- and zooplankton, fish). The novelty of the study is to add appropriate equations for endocrine

disruption. In AQUATOX, reproductive endpoints exist for fish with different age- or size-classes. With regards to endocrine disruption, males have to be differentiated from females. Therefore, three sex-classes were first added in the model: juveniles, males and females. Intersex fish are not considered because they can still reproduce and the important endpoint for modeling endocrine disruption is the reproductive ability of fish. Instead, a reproductive factor is associated with adults. For example, a non contaminated fish has a 100% reproductive factor while an intersex fish has a lower value, depending on the contamination level. Further model development will allow predicting the percentage of intersex fish, juveniles, males, and females. In addition to modeling the direct effects of EE2 on fish, the ecosystem model considers the lake dynamics (biomass, physico-chemical properties, hydraulics, etc.) and EE2 biomagnification in the aquatic food web.

**561.1 DEBtox based TK/TD model integrated with Matrix models for mechanistic interpretation of the combined effects chemicals and environmental stressors**

N.T. Hamda, Jagiellonian University / Institute of Environmental Sciences, Jagiellonian University / Environmental Sciences; D. Jevtic, R. Laskowski, Jagiellonian University / Institute of Environmental Sciences. Ecological risk assessment is aimed at predicting adverse effects of chemicals on natural populations. The information on the ecotoxicity is derived from laboratory bioassays in which individual-level effects of toxic chemicals on survival, fecundity, and body growth rate are measured. These bioassays are standardized and are conducted under constant and favorable conditions. However, organisms typically are found in environments that are neither constant nor optimal. Thus, the translation of results of standard toxicity tests to field situations is difficult because many factors are involved. It is also economically prohibitive or just impossible to experimentally assess all possible interactions. Thus, we need tools that can help to understand combined effects of environmental and chemical stressors on individuals and further assist us in assessing effects on populations in their natural environments. In this study, we combined DEBtox framework based TK/TD model with population dynamics models for the mechanistic interpretation of the combined effect heavy metals and external ecological stressors (temperature & density dependence) upon *Folsomia candida* population, a standard species for risk assessment of soil invertebrates. The modelling technique outlined here allowed us to interoperate the combined effect of chemicals and key ecological factors at individual-level mechanistically. Further the technique enhances the applicability of age and stage based matrix models to extrapolate these individual-level effects to the population level. Our model results prevail that the 20°C OECD based standard toxicity test underestimates both the individual and population level effects of Cd on *F. candida*. Exclusion of density dependence factor in extrapolating effects from individual-level to population level might overestimate or underestimate effects at population-level.

**562 A Mass Balance for Natural Estrogens on a Swine Farrowing CAFO**

E. Yost, North Carolina State University / Environmental and Molecular Toxicology; M.T. Meyer, United States Geological Survey / Organic Geochemistry Research Laboratory; B. Lee, Duke University / Nicholas School of the Environment; S.W. Kullman, North Carolina State University / Environmental and Molecular Toxicology. The waste from concentrated animal feeding operations (CAFOs) is known to contain appreciable quantities of steroidal estrogens, which are naturally produced by livestock and eliminated in their excreta. Here, we take a mass balance approach to track the fate of these compounds within the waste management system of a swine farrowing CAFO, which houses 5000 pregnant and lactating female swine. Analysis of estrogens was made in relation to: 1) estrogen excretion by individual animals; 2) the stability of estrogens in open pit holding lagoons during waste storage; and 3) mobility and attenuation of estrogens following sprayfield application of swine waste as fertilizer. LC/MS-MS results indicate that the excretion of natural estrogens by the swine increases dramatically during the late stages of pregnancy, highlighting the importance of sow reproductive status in determining the estrogen load of the waste. Estrogen output per volume of waste ranged from  $>1\text{ ng/l}$  to  $27\text{ }\mu\text{g/l}$  in sow urine, and  $>1\text{ ng/kg}$  to  $428\text{ }\mu\text{g/kg}$  in sow feces, with a defined gradient of increased estrogen output associated with advanced stages of pregnancy and lactation. During storage in the lagoon, the natural estrogens contained in the raw swine excreta (e.g.  $17\beta$ -estradiol,  $17\alpha$ -estradiol, estrone, estriol) appear to undergo a biotic and/or abiotic transformation, resulting in the emergence of estrone as the predominant form of natural estrogen in the lagoon. Total estrogen concentrations in the lagoon wastewater average  $9.2\text{ }\mu\text{g/l}$ , with concentrations remaining relatively constant across all sampling seasons. Due to sorption of estrogen to solids in the lagoon, concentrations of estrogen in the lagoon sludge are elevated ( $\sim 20$ -fold higher) compared to concentrations in the lagoon wastewater. Following the land application of lagoon wastewater to sprayfields, the natural estrogens are attenuated relatively quickly (half life = 3.3 days); however, estrone was found to persist in soil at parts-per-trillion concentrations for up to 2 months following the land application. An expected outcome of this project is an increased understanding of the operational practices that affect the fate of estrogens on a swine CAFO, and prioritization of practices that may mitigate the off-site transport of these compounds.

**563 Dissipation of three veterinary antimicrobials in stockpiled feedlot manure**

A.J. Cessna, National Water Research Institute / Environment Canada, Environmental Health National Program / Agriculture and Agri-Food Canada; S. Sura, Agriculture and Agri-Food Canada (AAFC) / Food and Bioproduct Sciences; D. Degenhardt, Alberta Innovates – Technology Futures; F.J. Larney, A. Olson, T.A. McAllister, Agriculture and Agri-Food Canada (AAFC). Large quantities of veterinary antimicrobials are used in animal production as a result of therapeutic uses to treat disease and sub-therapeutic uses to prevent disease and promote growth. Up to 80% or more of administered antimicrobials can be eliminated in the feces or urine either as parent compounds or their metabolites. Consequently, they are introduced into the environment mainly through application of manure as fertilizer to agriculture fields or through leaching or runoff from manure storage locations (stockpiles, windrows, lagoons). There is worldwide concern that environmental contamination by these chemicals may result in widespread bacterial resistance to antimicrobials used in human medicine. Aerated windrow composting has been shown to decrease antimicrobial concentrations in beef cattle (*Bos taurus*) manure; however, it is unclear whether concentrations would dissipate to the same extent if the manure was stockpiled. Consequently, a study was conducted at Agriculture and Agri-Food Canada Research Centre, Lethbridge, AB in fall 2010 to investigate the dissipation of three antimicrobials, commonly used in beef cattle production, in stockpiled manure. Beef cattle (10 steers per pen) were administered one of the following treatments in the research feedlot situated on the Research Centre: (1) 44 mg of chlortetracycline  $\text{kg}^{-1}$  feed (dry-weight), (2) 44 mg of chlortetracycline + 44 mg sulfamethazine  $\text{kg}^{-1}$  feed, (3) 11 mg of tylosin  $\text{kg}^{-1}$  feed and (4) no antimicrobials (control). Manure was collected from the feedlot pens and stockpiled (2 replicates per treatment = 8 stockpiles). Because the stockpiles were not regularly turned, manure samples were collected periodically from three locations (top, center, and bottom) within the stockpiles over a period of 140 d. The samples were extracted using accelerated solvent extraction and the extracts analyzed for chlortetracycline, sulfamethazine and tylosin using LC/MS/MS analysis. The antimicrobial

concentration data will be used to determine the dissipation kinetics of the three antimicrobials when beef cattle manure is stockpiled.

**564 Impact of animal manure separation technologies on steroid hormone distribution – consequences for agricultural practices**

M. Hansen, University of Copenhagen, Faculty of Health and Medical Sciences / Section of Advanced Drug Analysis, Toxicology and Environmental Chemistry Laboratory; O. Popovic, University of Copenhagen; E. Bjorklund, K.A. Krogh, University of Copenhagen, Faculty of Health and Medical Sciences; L.S. Jensen, C.S. Jacobsen, University of Copenhagen; B. Halling Sorensen, University of Copenhagen, Faculty of Health and Medical Sciences. When steroid hormones are emitted into the environment, they may have harmful effects on the reproduction system of aquatic life. Until now, research has primarily focused on human excretion, demonstrating that steroid hormones reach the aquatic environment due to insufficient removal in waste water treatment processes. However more recently, it has been revealed that agricultural practices also may add to the environmental burden of steroid hormones. So far, research activities have mainly focused on steroid estrogens, but also androgens, progestagens and glucocorticoids, expressed in the vertebrate steroidogenesis, may occur at substantial levels in animal manure and should be addressed. In agricultural practices the animal manure can be applied to the soil as raw manure, but also as a solid or liquid manure fraction, since current livestock production facilities utilizes a recently developed technology, which separates raw animal manure into a solid and a liquid fraction. This technology offers an improved handling and refined distribution of the manure nutrients to the farmlands and the possibility to reduce the environmental impact of manure nutrients, especially avoiding the surplus load of phosphorous. In the present work we investigated the distribution of 9 steroid hormones (pregnenolone, progesterone, dehydroepiandrosterone, androstenedione, testosterone, dihydrotestosterone, estrone,  $17\alpha$ -estradiol and  $17\beta$ -estradiol) in raw manure and manure separates from 10 to 15 different pig farms in Denmark utilizing 4 different separation technologies. Furthermore, we investigated a possible relationship between the steroid hormone concentration and the different manure fractions and separation technologies. The chemical steroid hormone analysis was done by inverse and integrated clean-up pressurized liquid extraction, and further cleaned by a two step solid-phase extraction before derivatization and finally analyzed by GC-MS/MS. It was found that the steroid hormones were predominant in the solid manure separate calling for manure management strategies to reduce the content of steroid hormones in separated manure solid fraction. This could potentially be achieved through composting or anaerobic digestion for biogas production of the solid fraction; however, the effects of these technologies on steroid hormones need to be verified.

**565 Occurrence of polybrominated diphenyl ethers (PBDEs) and 2,2',4,4',5,5'-hexabromobiphenyl (BB-153) in landfill leachate in Cape Town, South Africa**

A.P. Daso, Cape Peninsula University of Technology, Cape Town, South Africa / Environmental and Occupational Studies, Cape Peninsula University of Technology / Food and Agricultural Sciences; O.S. Fatoki, Cape Peninsula University of Technology, Cape Town, South Africa / Department of Chemistry; J.P. Odendaal, Cape Peninsula University of Technology, Cape Town, South Africa / Department of Environmental and Occupational Studies. An assessment of the concentrations of selected PBDE congeners as well as BB 153 in leachate samples collected from three landfill sites within the City of Cape Town was conducted. A liquid-liquid extraction technique was employed for the isolation of all the target compounds from the leachate samples. Extracts obtained were further subjected to multilayer column chromatography employing different forms of silica gel. The prepared samples were analysed using a high capillary gas chromatograph equipped with a micro-electron capture detector (GC- $\mu$ ECD). The overall mean concentrations of the total PBDEs, including BDE 209 ranged between 5.65 – 2243.93, 0.28 – 20.51 and 1.66 – 1169.53 ng/L for Bellville, Coastal Park and Vissershok landfill sites, respectively. The mean concentrations of BB 153, which were generally low in most of the samples analysed, were 70.35, 7.14 and 8.16 ng/L for Bellville, Coastal Park and Vissershok sites, respectively. The influence of precipitation on the characteristics and quantity of leachate produced from the landfill sites investigated was most pronounced during the August/September sampling regime. Generally, the trend observed in this study clearly indicated a wide variation in the levels of these contaminants in all the landfill sites studied from one sampling period to the other. However, the principal component analysis revealed that release of these contaminants might be associated with two or



three possible sources. This study further confirmed the relevance of landfill leachate as an important source of PBDE contamination of the environment, especially the groundwater and surface water sources.

**566 Photochemical Degradation of Pharmaceutical Compounds in Wastewater Effluents** F. Rosario-Ortiz, University of Colorado; M. Dong, University of Colorado Boulder. The photochemical fate of pharmaceuticals in wastewater effluents is of interest as these effluents constitute one of the most important sources of these compounds into the environment. The photochemical degradation of five pharmaceuticals was studied in two secondary wastewater effluents (WWA and WW B) collected over two sampling events (February and March of 2012). The samples were exposed to direct sunlight for a total of five days and collected daily for analysis. The pharmaceuticals evaluated were atenolol, carbamazepine, meprobamate, phenytoin and primidone. The overall degradation of these pharmaceuticals was dependent on both direct and photosensitized photolysis. The contribution of each process was determined by the chemical properties of the compounds. For the photolysis tests, the contribution of direct photolysis was evaluated by exposing the samples to sunlight in buffered lab water. Photosensitized degradation was evaluated by quantifying the degradation of the compounds in wastewater. In photosensitized degradation, the concentrations of the hydroxyl radical (HO•) and singlet oxygen (<sup>1</sup>O<sub>2</sub>) were quantified using the hydroxylation of benzene and degradation of furfuryl alcohol, respectively. No significant direct photochemical degradation was observed for all five compounds investigated during time of exposure. After a 5 day exposure, the photosensitized degradation of the compounds ranged from 69 to 91% for atenolol, 67 to 98% for carbamazepine, 16 to 52% for meprobamate, 44 to 85% for phenytoin, and 34 to 88% for primidone in both wastewaters. The large variation in removal could potentially attribute to the difference in their reactivity with oxidants generated in the wastewaters. The average steady state HO• concentrations were 1.2

**567 Removal of Perfluorochemicals and Personal Care Products in Drinking Water Treatment Processes** Y. Tsai, G. Wang, C. Chen, National Taiwan University / Institute of Environmental Health. The issues of using reclaimed water are getting important because of water shortage; however, the information on eliminating emerging contaminants from drinking water treatment is still limited. This study focused on the efficiency of conventional and ultraviolet (UV) treatments to remove eight perfluorochemicals (PFCs) and 27 personal care products (PCPs). Raw water spiked with three levels of the analytes were investigated on processes with laboratory scales: pre-chlorination, coagulation/sedimentation, rapid filtration, post-chlorination, and UV radiation. Water samples were adjusted to pH 3.0 then went through disk-type solid-phase extraction (SPE). The analytes were eluted with 0.1% ammonium hydroxide in methanol and 50% methanol/50% dichloromethane (v/v). The eluents were concentrated by a SpeedVac and were analyzed by ultra-high performance liquid chromatography/tandem mass spectrometry. Chromatographic separation were done at a flow rate of 0.5 mL/min with aqueous mobile phases of 5 mM ammonium acetate and 10 mM *N*-methylmorpholine, respectively, for basic and acidic analytes. Most PFCs were removed at 9-81% by coagulation/sedimentation; the rapid filtration eliminated about 40-90% of the PFCs but the effects of chlorination were very minor. Over 45% of most PFCs were eliminated through the whole procedure; nevertheless, PFCs did not degrade after UV radiation. Over 75% of most PCPs were degraded after the treatments of pre-chlorination or post-chlorination except for DEET and caffeine (lower than 10%), but the coagulation/sedimentation process did not have significant removal on PCPs. The rapid filtration step took out over 80% hormone compounds but only over 20% of analgesics. Most of the PCPs could be removed for over 80% through the whole treatment procedure as well as after the UV radiation.

**568 Survey of Emerging Contaminants in Biosolids Treated under Aerobic and Anaerobic Conditions** K. Dasu, Purdue University / Department of Agronomy, Crop, Soil and Environmental Sciences, NRC Post Doc at US EPA / Office of Research and Development, National Risk Management Research Laboratory, U.S. Environmental Protection Agency / National Risk Management Research Laboratory; M.A. Mills, U.S. EPA, U.S. EPA / National Risk Management Research Laboratory, ORD, US EPA / Remediation and Pollution Control Division, NRMRL, U.S. EPA / Office of Research & Development. Emerging contaminants (ECs) are detected globally in wastewater treatment plant (WWTP) effluents and biosolids. These

matrices may play an important role in the transport of these contaminants into the environment. There is a growing concern about the potential risks associated with the land-application of biosolids to the aquatic ecosystems and humans, as many of the ECs are shown to have endocrine disrupting properties and other harmful effects. Once land-applied, the emerging contaminants present in biosolids can enter the surface and ground waters or carry over to the plants grown on such soils and this increases the potential exposure of humans and aquatic ecosystems to these compounds. The main objective of the current study is to evaluate the occurrence, concentrations and composition of ECs and their precursors or degradation products in different biosolids treated under aerobic and anaerobic conditions. Classes of chemicals to be monitored include perfluorinated chemicals and their precursors and total fluorine content in the surveyed biosolids, alkylphenol ethoxylates and alkylphenols, steroid hormones and pharmaceuticals and personal care products, along with metals and pathogens. The data will aid in understanding the fate and transport of ECs in wastewater matrices and in the assessment of the risk associated with the land-application of biosolids. The biosolid samples treated under aerobic and anaerobic conditions at the surveyed WWTPs are extracted and analyzed separately for all the selected ECs. A grab sample of effluent from the same WWTPs is also analyzed. From the current occurrence study, the data on the concentrations and composition of different ECs and their precursors or metabolites in biosolids treated under different conditions will be discussed in detail.

**569 The degradation of antidepressant pharmaceuticals in aerobic municipal wastewater sludge** M.M. Schultz, College of Wooster / Department of Chemistry; M. Murphy, K. Sullivan, S. Strand, M. Snider, The College of Wooster. Public and regulatory interest regarding the presence of pharmaceuticals and personal care products (PPCPs) in the environment is increasing. Human pharmaceuticals may enter into the environment through wastewater treatment plants by domestic waste from human excretion or direct disposal of drugs in toilets. Many of these untreated PPCPs may enter aquatic and terrestrial environments and negatively impact metabolic pathways of the ecosystem's organisms. Within wastewater treatment there are many routes by which chemicals are transformed; however, biodegradation due to the metabolic activities of microorganisms during the treatment process is the predominant route of PPCP breakdown. Consequently, it may be possible to decrease the amount of biologically active PPCPs entering the environment by augmenting WWTPs with microbes capable of more efficiently degrading these molecules. However, few microbes with these abilities have yet to be identified; therefore, this is an important area for discovery. To establish a model system for exploring the biodegradation of PPCPs, initial studies investigated the degradation of two PPCPs that are ubiquitous wastewater contaminants: sertraline and venlafaxine, both widely prescribed antidepressant pharmaceuticals. To learn whether microbial communities in the wastewater sludge environment have the ability to degrade sertraline and venlafaxine and, thus, consequently reduce their environmental loads, microcosms containing aerobic sludge were spiked with deuterated sertraline and venlafaxine. Samples collected over 36 days were analyzed for the depletion of the added pharmaceuticals by microscale solvent extraction followed by liquid chromatography tandem mass spectrometry. Municipal aerobic wastewater sludge was found to degrade sertraline and venlafaxine by over 90% over the course of 36 days, whereas no change in the added sertraline and venlafaxine concentrations were observed in abiotic control microcosms. Two venlafaxine metabolites, *o*-desmethylvenlafaxine and *n*-desmethylvenlafaxine, were observed as early as day 2 of the microcosm experiment. Further metabolite identification is underway.

**570 The Curve is the Measure of Toxicity** W.G. Landis, Western Washington University / Institute of Environmental Toxicology, Western Washington University / Institute of Environmental Tox. & Chem.; D.R. Moore, Intrinsik Environmental Sciences, Inc. (US). The foundational principle of environmental toxicology is that the exposure-response curve describes the interaction between a toxicant and the endpoint of interest. A number of papers since the 1940s have demonstrated that curve fitting as the best description exposure response. So it has been established the dominant paradigm is that curve fitting is the best description of the exposure-response relationship. The response can be mortality, reproduction, induction of enzymes, changes in species composition in a microcosm, or alteration in an ecosystem service. The assumption of the exposure-response curve as the fundamental model of toxicity means that experimental data should be

taken that describes the entire curve. There are a number of computational tools that can be used to describe the parameters of the curve ranging from programs in R to Bayesian curve fitting algorithms. If predicted by QSAR, extrapolation from molecular interactions or data from tests from other species, the exposure-response curve is a testable hypothesis. The slowness of the adoption of curve fitting as the primary method of reporting exposure-response appears to be a cultural, not scientific issue.

**571 NOECs are Needed** J.W. Green, DuPont / Applied Statistics Group; T.A. Springer, Wildlife International, Ltd. The conceptual advantages for ECx estimates from regression models fit to concentration-response data from toxicity experiments are clear. Among the advantages cited for the use of ECx estimates in risk assessment is that there is no need for post analysis discussion of the biological significance of statistically significant differences between control mean and treatment mean responses. It is also often suggested that ECx estimates will identify biologically important effects that can be missed by an analysis to determine a NOEC because the effect may not be large enough to result in statistically significant differences. Criticisms of NOECs have been pointed out in numerous papers. It is the goal of this presentation to present evidence that the criticisms of NOECs and the advantages of ECx estimates have both been exaggerated. While ECx estimates are certainly valuable, even preferable, under the right conditions, those conditions do not always exist and it is foolish to call for the removal of an important tool from the statistician who must analyze a wide range of responses from many types of ecotoxicity experiments. Appropriate statistical models and tests will be presented to demonstrate that hypothesis testing has much more to offer than is indicated by the performance of suboptimal techniques often presented as evidence for why NOECs should be discarded or even banned. These will include types of responses for which no regression models have so far been proposed or accepted within the ecotox community. Some recent criticisms of NOECs suggest a need to make the ecotox community more aware of the advances in multiple comparison methods relevant to ecotox studies over the last 15 – 20 years and this talk will attempt to rectify that. Furthermore, limitations on regression estimates will be discussed which make them unsuitable for some datasets where one would expect ECx to be a viable approach. In addition, some realities of GLP experiments to meet regulatory guidelines appear not to be appreciated.

**572 Just Say No to NOELs** D.R. Moore, Intrinsik Environmental Sciences, Inc. (US); W.G. Landis, Western Washington University. Several recent articles and editorials have recently appeared in the literature arguing for the abolition of NOELs and LOELs in ecotoxicology journals. The goal would be to replace these hypotheses testing endpoints with regression-based ECx values. There are a number of reasons why NOELs and LOELs have been criticized, most of which date back over 25+ years: (1) the NOEL and LOEL are always treatment levels and no correspond to consistent effect levels, (2) poor experimental design will mistakenly indicate that a substance is less toxic than it really is, and (3) most of the information from the toxicity test is not used and thus the investigator has no means of determining confidence in the estimated effects metrics or using the results to estimate risks of differing severity. The alternative is to use a regression-based approach to generate concentration-response relationships. A variety of easy-to-use software packages can be used to conduct such analyses. Further, the regression-based approach is highly flexible and can be used for quantal, count or continuous response variables. It can also be used to estimate toxicity thresholds (e.g., using a hockey stick model) or account for hermetic responses. Thus, there is no reason for continuing to use NOELs and LOELs in ecotoxicology. In this talk, we will illustrate the ease and flexibility of the regression-based approach for estimating effects metrics.

**573 Problematic Responses from Ecotoxicity Experiments** T.A. Springer, Wildlife International, Ltd.; J.W. Green, DuPont / Applied Statistics Group,. The advantages of ECx estimates relative to use of the NOEC in expressing results of laboratory ecotoxicity experiments for endpoints such as length, weight, and mortality, where data are collected from studies designed to support regression analysis, are well known. Recognition of these advantages has led some authors to call for a ban on reporting of NOEC values, and use of hypothesis testing in general, for all ecotoxicity studies. However, proponents of this ban fail to consider that there are types of responses that are not amenable to regression analysis to determine an ECx value, that there are some risk assessment questions that are as well or better

answered by providing a NOEC value, and that there are experiments that cannot be practically designed to support regression analysis. This presentation will demonstrate why a much broader consideration of the merits of reporting ECx values instead of NOEC values is needed, and why a ban on use of NOECs and hypothesis testing is not advisable. Four classes of responses that arise in ecotoxicity experiments that present challenges to statistical analysis will be discussed. For these classes of responses, generally accepted methods for calculating ECx values are unavailable, and often, a meaningful ECx cannot be estimated. Indeed, it is not always clear what the meaning of an ECx value would be for the responses in question. On the other hand, statistical methods (typically based on trend analysis) that can identify a meaningful and protective no observed effect concentrations (NOEC) are available. Use of modern methods for the derivation of NOEC values will be compared to use of regression methods for determining ECx values for (1) severity scores that arise in histopathology assessments of fish studies to detect endocrine disruption [e.g. OECD TG 229], (2) analysis of serum vitellogenin (VTG) concentrations measured in the fish short term reproduction assays [OECD TG 229], (3) analysis of first day of egg hatch in fish early life stage tests [OECD TG 210], and (4) sex-ratio in the fish sexual development test [OECD TG 234]. In each case, the rationale for use of NOEC values instead of ECx values in a regulatory/risk assessment context will be explored.

**574 EPA's Test of Significant Toxicity: Impact on the Permit Compliance of Honolulu's Wastewater Treatment Plants** L.C. Vazquez, C&C of Honolulu / Department of Environmental Services. The U.S. Environmental Agency has developed an alternative statistical approach, based on bioequivalence, to address the limitations associated with the use of No Observed Effect Concentration (NOEC) for interpreting whole effluent toxicity (WET) data. The traditional hypothesis testing may deem small effects with no environmental consequence as significant or fail to detect unacceptable toxicity when data precision is low. The test of significant toxicity (TST) corrects these anomalous conclusions by determining whether there is a biologically relevant difference between the effluent at the critical concentration and the control and by integrating both Type I and Type II error rates in the statistics. This new approach is under consideration by the State regulatory agency for inclusion in permits issued to Hawaii dischargers. Such change in the permits could affect the City & County of Honolulu's regulatory compliance. The City operates four wastewater treatment facilities that are required to monitor effluent toxicity with EPA chronic protocols using *Ceriodaphnia dubia* and a local sea urchin, *Tripneustes gratilla*. Data sets from 820 tests meeting acceptability criteria, collected over a five year period of WET monitoring of the City's treated discharges, were analyzed using the NOEC method and the TST approach. The NOEC method, even when subjected to percent minimum significant difference limits-based criterion, declared a remarkably greater number of toxic incidences particularly with the more sensitive urchin fertilization method. On the other hand, the TST approach found toxic effects in *C. dubia* reproduction data from some effluent samples that the NOEC method declared acceptable. Conclusions derived using these two statistical approaches on WET data from wastewater facilities with different levels of treatment will be compared.

**575 Biological arguments for selecting ECx values in chronic aquatic toxicity testing** C. Mebane, US Geological Survey / NOAA Fisheries Liaison, US Geological Survey/WRD / NOAA Fisheries Liaison. A fundamental purpose of chronic toxicity testing is often to predict "safe" levels of a substance in sustained exposures for organisms in aquatic environments. Early-life stage and other chronic toxicity testing customarily have been statistically evaluated through hypothesis testing to estimate no-observed-effects concentrations (NOECs) and lowest-observed-effects concentrations (LOECs). Hypothesis testing has been roundly criticized as being inferior to regression-based approaches for making point estimates of specified levels of effect (ECx). However, the selection of just what levels of effect (x) would be acceptable has sometimes followed circular reasoning using comparisons back to NOECs or LOECs rather than considering what ECx value would likely be sustainable in populations or communities in the wild. Effects studied in early-life stage toxicity testing with aquatic organisms (reduced growth, fecundity and survival) were reviewed in the context of responses of wild fish populations to disturbances associated with changes in mortality or growth rates. The review suggests that different ECx values would be appropriate for different endpoints and for species with different life histories. With some temperate, freshwater fish, differences in size (as length) of as



little as 5% can determine inter- or intra-specific contests which in turn may disproportionately determine survival for contest winners or losers. Growth reductions of the magnitude of 20% could predict extremely high indirect mortalities for juvenile fish. In contrast, under strong density dependence such as that often occurring in temperate stream-resident salmonid populations where juveniles have to compete for limited shelter to survive their first winter, much greater than 20% loss of the young-of-year is routinely absorbed. These observations suggest, for example, that when interpreting lethal and sublethal effects from toxicity test data, an EC20 for fecundity or first-year survival in density-dependent fish populations would conceptually be sustainable, yet for reduced growth (as length of juvenile fish) an EC5 would be a more appropriate endpoint.

**576 Bioaccumulation of gold nanomaterials by *Manduca sexta* through dietary uptake of surface contaminated plant tissue** J.D. Judy, University of Kentucky; J.M. Unrine, University of Kentucky, University of Kentucky / Department of Plant and Soil Sciences, University of Kentucky / Department of Plant & Soil Sciences, University of Kentucky / Savannah River Ecology Laboratory, University of Kentucky / Department of Plant and Soil Sciences, University of Georgia / Savannah River Ecology Laboratory; P.M. Bertsch, University of Kentucky. Mass loading of soil particles by wind, rain, biota, and/or mechanical disturbance onto plant surfaces is a pathway by which contaminants can potentially enter terrestrial food webs. Previously, we demonstrated trophic transfer and biomagnification of manufactured nanomaterials (MNM)s from tobacco plants (*Nicotiana tabacum*) to a terrestrial secondary consumer, tobacco hornworm (*Manduca sexta*). In this study, we investigate the potential for bioaccumulation of MNMs from contaminated plant surfaces by tobacco hornworm caterpillars and compare the results of direct dietary exposure with the results of our previous trophic exposure study. We hypothesize that the caterpillars will not bioaccumulate as large a mass of MNMs through direct exposure as they did through trophic exposure. Caterpillars were fed tomato leaf tissue that had been surface contaminated with 12 nm tannate coated gold (Au) MNMs. Caterpillars received a dose of up to 3 µg of Au MNMs over 7 d. After dosing was complete, bulk Au concentrations in individual caterpillars were measured using inductively coupled plasma mass spectrometry (ICP-MS) after 0, 1, 4, and 7 d of depuration. Growth, mortality, and ingestion rate were monitored over the 14 d exposure. Spatial distribution of Au in caterpillar tissue samples was determined using scanning x-ray fluorescence microscopy (µXRF). No conclusive evidence was found suggesting that ingestion of plant material surface contaminated with Au MNMs affected caterpillar growth, mortality, or ingestion rate compared to controls. We found that caterpillars bioaccumulate Au MNMs, but that the assimilation efficiency of bioaccumulation was low. The caterpillars accumulated a much smaller mass of MNMs in this study than in the trophic transfer study, supporting our *a priori* hypothesis. Caterpillars eliminate ingested Au MNMs rapidly from 0–24 h, but very slowly from 1 d to 7 d. The retention of Au MNMs in the caterpillar tissues after 1 d depuration was confirmed by µXRF. These data indicate that MNMs resuspended from soil onto plant surfaces are bioavailable to terrestrial consumers, a result with important implications for the potential risk of nanotechnology to ecoreceptors in terrestrial ecosystems.

**577 Behavior of fullerene nanoparticles (nC<sub>60</sub>) in the terrestrial environment: studies on the potential release of fullerenes from biosolids** D. Navarro, R. Kookana, CSIRO / Advanced Materials Transformational Capability Platform, Land and Water; M. McLaughlin, CSIRO / Land & Water, University of Adelaide / School of Agriculture, food and wine, CSIRO / Advanced Materials Transformational Capability Platform, Land & Water; J. Kirby, CSIRO / Advanced Materials Transformational Capability Platform, Land & Water. In recent years, there has been increasing interest on the fate of nanoparticles (NPs) in wastewater treatment plants. Indeed, among different stages involved in wastewater treatment, the presence of NPs in sludge materials has often been reported. With sludge being a potential sink for most NPs, the release of NPs from these sludge materials is still unknown. Application of biosolids (treated sludge) as soil amendments/fertilizers, hence, presents another important route for NPs to be remobilized and enter the environment. In this study, the release of fullerenes (C<sub>60</sub>) from biosolids spiked with different forms of fullerene nanoparticles (nC<sub>60</sub>) was examined across a wide range of soils. Results showed that different levels of C<sub>60</sub> could be released from different biosolid-soil systems, suggesting that soil properties influence the mobilization of C<sub>60</sub> in the terrestrial environment. The form of nC<sub>60</sub> used, on the other hand, had negligible effect on

the levels of C<sub>60</sub> detected in released fractions. Aging nC<sub>60</sub> in these systems only slightly increased the release of C<sub>60</sub>. Even after 8 w of aging, majority of the biosolid-soil systems only released approximately 3% of the initial nC<sub>60</sub> spike. The application of biosolids to soil favored the retention of C<sub>60</sub> rather than release. Though the exact release mechanism in these complex systems is still unclear, it is apparent from the data that both pH and natural organic matter played a part in controlling the binding and release behavior of fullerenes in soils.

**578 Examining single walled carbon nanotube distribution in live fish during gavage and feeding studies using near infrared fluorescence detection** J.H. Bisesi, Clemson University / Institute of Environmental Toxicology (CU-ENTOX), University of Florida / Department of Environmental and Global Health; J. Merten, Arkansas State University / Department of Chemistry and Physics; A.N. Parks, Duke University / Nicholas School of the Environment; L. Ferguson, Duke University / Department of Civil and Environmental Engineering, Duke University / Nicholas School of the Environment, Pratt School of Engineering / Department of Civil & Environmental Engineering; T. Sabo-Attwood, University of Florida / Department of Environmental & Global Health. Single-walled carbon nanotubes (SWNTs) are emerging as one of the most commercially important and technologically relevant nanomaterials in research and consumer industries. However, their potential release has raised concerns about their impacts on environmental health and safety. Carbon nanomaterials are challenging to detect in organic matrices; limiting our ability to assess systemic distribution and elimination in model organisms. Excitation of SWNTs with visible light produces a distinctive fluorescence emission in the near infra-red range. The use of near infra-red fluorescence (NIRF) is emerging as a highly sensitive and selective technique for accurate quantification, purification, and tracking of these materials in biological organisms, with robust discrimination against endogenous fluorescence. Using this technology, we have constructed a highly sensitive and selective custom NIRF imaging system for tracking SWNTs in fish. This system utilizes a 30 watt Visotek 808 nm laser for deep tissue excitation coupled with a Princeton Instruments InGaAs spectroscopy array detector. We have optimized this system to be able to detect SWNTs with real-time repeated measurements in live fish. For these studies, fathead minnows (*Pimephales promelas*) were gavaged with 2.5, 5, and 10 µg of SWNTs and imaged at 2, 4, 8 and 24 hours post exposure. Following each time point, replicate fish were euthanized and necropsied for imaging of individual organs as well as quantification of SWNTs using the Applied NanoFluorescence NS1 NanoSpectralyzer. Results of these studies reveal that SWNTs remained primarily in the GI tract and were undetectable after 8 hours. These data suggest that the majority of SWNTs do not get absorbed in the GI tract and are likely depurated. This was confirmed by the complete loss of fluorescent signal when the intestinal tract was removed. We also were able to quantify the SWNTs in homogenized intestines using near-infrared spectrometry. We have begun to assess the distribution of SWNTs in fish maintained on SWNT-containing feed stocks. The use of this NIRF imaging system is a powerful tool for assessing in vivo distribution and potential toxicological target sites for SWNTs in organisms. Data from these studies can lead to a more targeted approach in the understanding of the biological fate, mechanisms of action, and health impacts associated with SWNT exposures, which is invaluable in the assessment of risk for these materials.

**579 Nanoparticle Fate Assessment and Toxicity in the Environment – Initial findings from phase 1 of the NanoFATE project** C. Svendsen, Centre for Ecology & Hydrology, Monks Wood / Pollution and Ecotoxicology, NERC Centre for Ecology & Hydrology / Hails Section, NERC Centre for Ecology & Hydrology / Ecotoxicology; M. Diez, Centre for Ecology and Hydrology; A. Crossley, University of Oxford / Department of Materials; G. Cornelis, University of Gothenburg / Department of Chemistry and Molecular Biology; M. Hasselov, Goteborg University / Chemistry Dept; S. Loureiro, Universidade de Aveiro / department of Biology & CESAM, Universidade de Aveiro / Departamento De Biologia; S. Lofis, NERC Centre for Ecology & Hydrology / Shore Section; K. van Gestel, Vrije Universiteit Amsterdam / Institute of Ecological Science, Vrije Universiteit Amsterdam / Department of Animal Ecology; F. Dondero, University of Piemonte Orientale Amedeo Avogadro / Department of Science and Technological Innovation; A. Johnson, R. Williams, L. Walker, Centre for Ecology and Hydrology; D. Spurgeon, NERC Centre for Ecology and Hydrology. This presentation will outline the main data and findings from the first 2.5 years



of the EU FP7 project NanoFATE. The project is a multi-disciplinary effort to assess the environmental risks posed by engineered nanoparticles (ENPs) and test the applicability of current fate and risk methodologies and identify possible improvements required for application with ENPs. The work is undertaken using commercial ENPs from high-volume products for which recycling is not an option, e.g., fuel additive, personal care and antibacterial products (CeO<sub>2</sub>, ZnO, Ag of varying size, surface and core chemistries). These are being followed through their post-production life cycles i.e. from environmental entry as “spent product”, through waste treatment to their final environmental fates and potential toxic effects. In the initial phase, cross-disciplinary work has been used to determine how ecotox exposures (soil and aquatic) can best be set up and whether it is possible to follow current standard protocols. Material scientist, environmental chemists, ecotoxicologists, fate modellers and risk assessors have worked together to: 1) assess what a realistic exposure of ENPs is likely to be and how best to reflect this in hazard testing, and; 2) how spatial predicted environmental concentrations (PEC) estimates can best be made including demographic and geographical variations across Europe. Specific properties that need principal consideration during the development, adaptation and validation of environmental fate models for nanoparticles have been identified and prioritised. Based on this initial basic fate models have been completed and the initial worst case environmental concentration estimates been supplied serving to inform exposure design. While the main focus of the talk will be detailed fate and effects results from soil experiments looking at the consequences of soil chemistry and long term behaviour of ZnO and Ag ENPs on the uptake and ecotoxicological effects observed, it will also include the current risk assessment results for all 3 ENP types in both water and soil environments including example risk maps. The main problem areas encountered with applying current “standard chemical” fate and risk assessment methodology to ENPs during the project will be highlighted in the conclusions.

**580 Life-stage-dependent response in zebrafish (*Danio rerio*) to phototoxicity of TiO<sub>2</sub> nanoparticles** H. Ma, U.S. Environmental Protection Agency / Mid-Continent Ecology Division, Mid-Continent Ecology Division, USEPA; S.A. Diamond, U.S. EPA / MED, U.S. EPA / Mid-Continent Ecology Division, U.S. EPA / MED. The zebrafish, especially its embryo stage, has been increasingly used as a model to evaluate toxicity of manufactured nanomaterials. However, many studies have indicated that the chorion may protect developing embryos from the toxic effects of nanomaterials, suggesting that post-hatch life-stages may be more susceptible to nanomaterial toxicity and should continue to be tested to fully discern the potential hazard and risk of nanomaterials. The current study aimed to identify the “window of susceptibility” (the life stage that is most sensitive to toxicity) of zebrafish to phototoxicity of TiO<sub>2</sub> nanoparticles. To this end, 96-h toxicity tests were conducted to zebrafish at different life-stage (embryo [ < 2-h post fertilization, “yolk-sac” larvae [ < 48-h post hatch], “free-swimming” larvae [21-d post hatch], and juvenile [50-d post hatch]) by exposing test organisms to TiO<sub>2</sub> nanoparticles under simulated solar radiation (4 h within every 24 h during the 96-h exposure period). Toxicity endpoints included hatching rate, % malformation, and % mortality for embryonic test, and mortality only for all other tests. For zebrafish embryo, TiO<sub>2</sub> concentration at 1-20 mg/l caused no mortality, had no impact on 72-h hatching rate, but induced malformation in fish embryos/larvae by up to 20%. TiO<sub>2</sub> at higher concentration (up to 200 mg/l) caused mortality to fish embryo/larvae with a 96-h LC50 of 34.4 mg/l (95% CI: 24.5, 48.4), but had no impact on hatching rate, suggesting that mortality mainly occurred after hatching. The “yolk-sac” larvae had a 96-h LC50 of 20.3 mg/l (95% CI: 18.9-21.8) and the “free-swimming” larvae had a 96-h LC50 of 134.6 mg/l (95% CI: 103.7, 174.7). For juvenile fish, TiO<sub>2</sub> concentration up to 200 mg/l caused mortality less than 25% after 96-h exposure. The finding that newly hatched zebrafish larvae (i.e., < 48-h post hatch) are more sensitive than fish embryo in response to TiO<sub>2</sub> phototoxicity suggest that zebrafish embryo test may not always be appropriate to evaluate toxicity of nanomaterials and relying on embryo toxicity data alone may substantially underestimate the potential risk of nanomaterials. These results will be discussed relative to risk assessment for TiO<sub>2</sub> nanoparticles.

**581 Transcriptional-level response and sub-cellular modifications of cyanobacteria induced by nano-titanium dioxide exposure** C. Cherchi, Northeastern University / Civil and Environmental Engineering; A. GU, Northeastern University / Department of Civil & Env Engineering, Biotechnology Initiative Program, Department of Civil & Env Engineering

/ Biotechnology Initiative Program. Progress of nanoscience poses an urgent need for fundamental understanding of the potential environmental effects of engineered nanomaterials such as nano-titanium dioxide (nTiO<sub>2</sub>). nTiO<sub>2</sub> have already been detected in wastewater effluents and will likely impact aquatic ecosystems (e.g. algae). The objective of this study was to investigate the impact of sub-lethal concentrations of titanium dioxide nanomaterials (nTiO<sub>2</sub>) on the nitrogen status of *Anabaena* PCC7120 using transcriptional-level information of biomarker genes involved in global response (*ntcA*) and in nitrogen fixation- (*nifH*, *nifK*), nitrogen assimilation- (*glnA*, *all2934*) and nitrogen storage-specific pathways (*cphA*, *cphB*). Additionally, intracellular nitrogen metabolites (aminoacids) were also monitored along with gene expression trends, to reveal subcellular changes and their contributions to the regulation of nitrogen metabolism and dynamics of nitrogen accumulation. Results indicated nTiO<sub>2</sub> dose-dependent patterns in the expression of the majority of the genes studied. During the light cycle, exposure to nTiO<sub>2</sub> at higher concentration (10 mgTiO<sub>2</sub>/L) led to distinctive up-regulation in the gene expression, in comparison to control. Overall down-regulations of these nitrogen metabolism genes were seen at the end of the dark cycle as a result of the low cell metabolism and energy (ATP) levels. The dose-dependent production of metabolites (aminoacids) involved in the GS-GOGAT pathway of nitrogen assimilation and in the intracellular nitrogen storage suggests that other pathways involving aminoacids biosynthesis or degradation might be activated or repressed in response to nTiO<sub>2</sub> exposure. The increase in total proteins was also observed and found to be, for the most, both time- and nTiO<sub>2</sub> concentration-dependent, reaching as high as 50% increase (of the control) in the sample exposed to 10 mgTiO<sub>2</sub>/L. This overproduction of aminoacids and proteins might partially contribute to the synthesis of newly synthesized proteins needed to carry out stress response mechanisms likely induced by nTiO<sub>2</sub> treatment. In conclusion, the subcellular modifications observed and the unbalanced genetic regulatory mechanisms of cyanobacteria under the environmental perturbations generated by nTiO<sub>2</sub> exposure are likely to modify their intracellular C/N ratios, thus impact, at larger scale, ecological trophic interactions and food web dynamics within complex ecological systems.

**582 Impacts of nanomaterial chemistry on the genomic response of the immune system of rainbow trout** R. Klaper, University of Wisconsin-Milwaukee / School of Freshwater Sciences, University of Wisconsin-Milwaukee / Great Lakes Water Institute; D.A. Arndt, University of Wisconsin Milwaukee / Great Lakes Water Institute, University of Wisconsin Milwaukee / School of Freshwater Sciences; J.P. Crago, Great Lakes WATER Institute, University of Wisconsin-Milwaukee, / Student; J. Chen, F. Goetz, University of Wisconsin-Milwaukee. The immune system is the first line of response of a vertebrate to a foreign substance and protects the organism from damage and rids it of unwanted chemicals and materials. This project assessed the innate immune reaction of an aquatic model, the rainbow trout (*Oncorhynchus mykiss*) to manufactured carbon nanomaterials (fullerenes and nanotubes) that were either unfunctionalized or functionalized with various chemical groups. Gene expression of macrophage cells from the head kidney of the trout were evaluated using single biomarkers and a global genomic approach using a microarray specific for the trout immune system. Concentrations that did not induce cellular toxicity were evaluated to determine 1) if nanomaterials in general were equally stimulatory of the immune system 2) how changes in the core of the nanomaterial impacted its effects on immune related gene expression 3) whether changes in the surface chemistry impacted the immune related response and 4) the impact of dose of nanomaterials on genomic response. We examined cell viability as well as gene expression of genes associated with a pro-inflammatory or antiviral response in a well-studied trout macrophage primary cell culture system. There was a significant difference among different carbon nanotube based nanomaterials in their level of pro-inflammatory gene expression behavior and the dose at which they became stimulatory. Functionalization to create water soluble particles caused a variable effect. As we decrease the concentration of nanomaterials we begin to see differences in inflammatory responses in a dose-dependent fashion and in global gene expression studies we found that the type of immune response differed with nanomaterial functionalization where there is little overlap in the genes expressed among treatments. Dose of nanomaterial also caused differences in global gene expression patterns. This would indicate that the dose that ultimately enters the organism will be extremely important to determine potential immune responses. The goal of this research is to create a mechanism with which to test other nanomaterials, provide data to support ecological risk assessments, and ultimately

inform decisions as to which materials will be the safest to industrialize and use with respect to aquatic environments.

**583 Uptake routes of metal nanoparticles in earthworms** M. Diez, Centre for Ecology and Hydrology; C. Svendsen, S. Dave, Center for Ecology and Hydrology; C. Van Gestel, Vrije Universiteit Amsterdam. This study aims at quantifying the contribution of dermal and oral uptake of silver (AgNP) and zinc (ZnONP) nanoparticles in earthworms. With the greater production and incorporation into consumer products, it is expected that increasing amounts of NPs will be released to the environment. These NPs will end up in soil through wastewater and sewage sludge or may directly be deposited on land, thus being of environmental relevance for terrestrial ecosystems. To assess their risk in soil, besides their dissolution and speciation it is important to investigate the mechanisms by which the different metal species (single nanoparticles, aggregates and metal ions) interact with biological membranes and enter the body of the organisms exposed. Earthworms are representative test organisms for ecological risk assessment. They will be exposed to contaminants in soil both through the skin (dermal) and by ingestion (oral). To assess bioavailability of metal nanoparticles in soil to earthworms, it is necessary to know the contribution of each pathway. For that reason, experiments will be performed with the earthworm *Lumbricus rubellus*, applying oral sealing with medical histoacryl glue to block the ingestion of soil particles. Glued and unglued earthworms will be exposed to Lufa 2.2 soil spiked with AgNPs and ZnONPs for 7 days. An exposure of glued and unglued earthworms to soil spiked with AgNO<sub>3</sub> and ZnCl<sub>2</sub> will also be included to address the interaction of silver and zinc ions with earthworm tissues. By measuring total silver and zinc body concentrations at different sampling times in these exposure scenarios it will be possible to assess the contribution of the different routes of exposure.

**584 Helping Contaminants Emerge: P & B Commercial Chemicals, Pharmaceuticals, Starting Materials, Impurities, By-Products and Degradation Products** P.H. Howard, Syracuse Research Corporation / Environmental Sciences Center; D. Muir, Environment Canada / Aquatic Ecosystem Protection Research Division, Environment Canada / Aquatic Contaminants Research Division. The Great Lakes and other North American regions have always been in the forefront in terms of identification of new environmental contaminants, particularly persistent (P) and bioaccumulative (B) organic chemicals. However, the identification has been by detailed analytical chemistry and is focused largely on structural analogs of previously identified compounds with only limited application of "in silico" approaches. Of particular concern are those substances that are P and B as defined by screening criteria for persistent organic pollutants (POPs). Such chemicals can move off shore from urban source regions and accumulate in waters and sediments of the open lakes and rivers where they can result in exposures of top predator fish and fish-eating birds. With improved instrumental capabilities, the past 10 years has seen a large number of new chemicals identified, mainly halogenated organics. However, the chemicals identified represent only a small fraction of the organic chemical substances in commerce. We have identified over 600 P&B chemicals using quantitative structure-activity relationships (QSARs) and expert judgment from a database of 22,000 commercial chemicals used in North America. In addition, we identified 58 High Production Volume (HPV) pharmaceuticals as well as 364 pharmaceuticals of unknown production volume that are potentially P&B and have not been detected in the environment. Presently, we are examining starting materials, impurities, by-products, and degradation products/metabolites of the P&B commercial chemicals that may be P&B chemicals themselves. Most commercial chemicals are synthesized from starting materials and reagents and result in the desired product plus leftover starting materials and by-products. These leftover starting materials or by-products can be environmental contaminants just like the desired commercial products. By reviewing the commercial synthetic pathways, we were able to identify possible leftover starting materials, impurities, and by-products. In addition, once a commercial chemical is used, it may be released to the environment in a variety of ways. Using available software and databases, we were able to predict potential degradation products/metabolites. Examples of impurities, by-products, and degradation products as well as some prediction successes (chemicals predicted to be P and B for which measurements has subsequently shown to be present) will be presented.

**585 Identification of unknown persistent halogenated compounds in environmental samples** E. Reiner, University of Toronto; L. Shen, Brock University; A. Muscalu, K. Jobst, A. Boden, P. Helm, Ontario Ministry of the Environment; C. Marvin, E. Sverko, Environment Canada; I. Brindle, Brock University. There are currently over 100,000 industrial chemicals and chemicals of commerce in use today[1]. Many of them as well as their degradation products can find their way into the environment. Due to limitations in analytical methods and technology, most labs currently monitor only a small fraction of these compounds which include the persistent organic pollutants (POPs) that are targeted under international treaties like the Stockholm agreement [2]. The majority of POPs are halogenated compounds that are persistent, toxic and bioaccumulative. Many of them as well as their degradation products undergo long range transit to remote areas where they were not manufactured or used. Mass spectrometry has been used as an analytical tool for 100 years, but not as a common environmental technique until the last 2 decades. High resolution mass spectrometry (HRMS) is an excellent tool for identifying unknown compounds groups that do not contain congeners or isomers. Unfortunately, environmental samples contain many thousands of compounds therefore requiring chromatographic separation to obtain pure mass spectra. Techniques like multidimensional gas chromatography (GCxGC) can greatly enhance analytical selectivity by resolving isomeric compounds enabling pure mass spectra to be obtained. The procedures involving advanced analytical techniques like GCxGC, HRMS and Ultra HRMS to identify halogenated POPs and their degradation products will be discussed.

**586 A non-targeted GCxGC/TOF-MS method, data analysis tools, and data reporting system for the advancement of environmental monitoring** E. Hoh, San Diego State University / Graduate School of Public Health; N. Dodder, SCCWRP; K.A. Maruya, Southern California Coastal Water Research Project, SCCWRP. Analytical methods for environmental contaminant monitoring are generally targeted. Contaminants that are either unknown or those not usually considered for analysis will not be observed by these targeted methods. Humans and wildlife may therefore be exposed to these unknown or uncommon contaminants without warning. In environmental contaminant monitoring, the ideal analytical method should concurrently determine both the known contaminants from the large number of possibilities, and have the ability to identify unknown contaminants. Comprehensive two-dimensional gas chromatography coupled with time of flight mass spectrometry (GCxGC/TOF-MS) is superior to conventional gas chromatography/mass spectrometry (GC/MS) for certain applications, particularly those which require the chromatographic separation of mixtures of similar compounds and/or the identification of compounds from their full-scan mass spectra. We developed a GCxGC/TOF-MS analytical method to identify and document unrecognized and novel marine contaminants in dolphins, a high trophic level sentinel species. The method characterized a broad array of non-polar, persistent and bioaccumulative contaminants. More than 130 uncommon contaminants, which are potentially bioaccumulative, were identified in addition to the typically monitored persistent organic pollutants. Due to the volume and complexity of the identification data, it was necessary to develop custom data organization/analysis software to document the evidence for the identifications and provide a mechanism for data sharing. We applied the non-targeted analysis to two ecotypes of bottlenose dolphins (coastal vs. offshore) and will describe the contaminant identification process for group of samples. Finally, we will discuss how non-targeted analysis may be incorporated to environmental monitoring networks to advance their effectiveness.

**587 Highly sensitive non-targeted screening for anthropogenic and naturally produced organohalogen contaminants using quadrupole systems** N. Rosenfelder, National Institute of Standards and Technology / Hollings Marine Laboratory; W. Vetter, University of Hohenheim; J. Kucklick, National Institute of Standards and Technology / Hollings Marine Laboratory. A large number of persistent and bioaccumulative organohalogen compounds of anthropogenic and natural origins are widely distributed in marine environments. These compounds pose a global threat to the environment and their thorough determination is an important task in environmental science. Most analyses of organohalogen compounds in environmental samples focus only on specific groups of substances while unknown or non-targeted compounds are overlooked or ignored. Comprehensive gas chromatography coupled to time of flight mass spectrometry (GC x GC TOF-MS) has been suggested as an alternative method. However,

this technique is currently not available to most laboratories. For this reason, we recently developed an alternative approach using a simple quadrupole system which is virtually found in all analytical laboratories (Rosenfelder et al., Anal. Chem 82 (2010) 9835-9842). The lower sensitivity of this method was overcome by switching to the selected ion monitoring (SIM) mode. The resulting GC/electron ionization-quadrupole-MS-SIM method (GC/EI-Q-MS SIM) was based on the fact that the retention times of most organohalogen compounds correlate with their molecular mass on non-polar GC-columns. Since the most important structural information is derived from the molecular ion, we screened only the high mass range ( $> 300$  u) in the SIM mode with  $\Delta u = 1$ . The method provided very high sensitivity and excellent mass spectra. It also featured an identification scheme for unknown organohalogen compounds. The benefits of this method were explored by the thorough analyses a commercial salmon sample which originated from a region with no direct persistent organic pollutant (POP) sources. Over 20 compounds with a molecular weight  $> 300$  u could be detected while only  $\sim 10$  could be detected in the full scan chromatogram of GC coupled to electron capture negative ion mass spectrometry (GC/ECNI-MS). Furthermore, the GC/EI-Q-MS SIM method provided univocal mass spectra down to 5 pg/compound and in most cases the molecular ion could be detected. PCBs, HCHs and other legacy POPs were only in low abundance. Instead, the most abundant compound in the sample was the naturally produced mixed halogenated monoterpene MHC-1. In addition, several unknown polyhalogenated compounds were detected in the early retention time range by GC/EI-Q-MS SIM. The structures and their toxic impact are currently unknown.

**588 Target and non-target screening using HPLC-Orbitrap to evaluate the contamination pattern along the River Rhine** M. Ruff, Eawag – aquatic research / Environmental Chemistry; H. Singer, M. Mueller, M. Loos, E. Schymanski, Eawag – aquatic research; J. Mazacek, AUE Basel; J. Hollender, Eawag – aquatic research. The Rhine is one of the most important rivers in central Europe, with 58 million inhabitants living in the catchment area. The river is used intensively economically and is a major source of drinking water following bank filtration. An international network of monitoring stations along the river permanently observes the river water quality. Besides their long-term monitoring activities, the stations focus on the detection of accidental spills, which requires powerful techniques for comprehensive daily analysis and real-time processing of the datasets. Organic micropollutants are subject to major political and public discussions, and tons of these chemicals daily enter the river via different sources. In the past, monitoring stations were mostly dominated by well-established gas-chromatographic MS techniques focusing on rather non-polar substances. As water is a strong polar solvent, this left a significant gap in water quality analysis. In order to address this deficit and be able to focus more on polar organic micropollutants, the implementation of liquid chromatography coupled to high resolution mass-spectrometry (LTQ-Orbitrap) techniques at the international monitoring station in Basel was supported. The adjustment of analytical methods and development of an automated data analysis tool (enviMass) allowed not only to extend the number of daily monitored chemicals to more than 300 but also to focus on the detection and prioritization of non-targeted chemicals. The data of a study where we followed a 7-day wave of the river Rhine over a distance of 900 km downstream, including the main tributaries, will be presented. A detailed spatial contamination pattern of hundreds of substances and unknown substances was obtained along the river. The load dynamics of targeted contaminations will be illustrated as well as regional sources of specific chemicals. With the non-target analysis, hundreds of unknown substances were prioritized along the river and a few of these could be identified and finally confirmed using standard compounds.

**589 Non-targeted analysis of emerging contaminants in wastewater and wastewater impacted aquatic environments** G.J. Getzinger, Duke University / Nicholas School of the Environment; L. Ferguson, Duke University / Department of Civil and Environmental Engineering, Duke University / Nicholas School of the Environment, Pratt School of Engineering / Department of Civil & Environmental Engineering. The identification of new and emerging contaminants in environmental media has been hindered by the need to utilize targeted mass spectrometric techniques, relying on the availability of analytical standards for each analyte. The emergence of high-resolution mass spectrometry (HRMS) and its application to the field of environmental chemistry has for the first time made possible identification

of emerging contaminants in complex environmental mixtures without *a priori* knowledge of contaminant identity or occurrence. In this study, we present a strategy and analytical workflow based on LTQ-Orbitrap Velos HRMS and MS/MS in conjunction with newly developed software (e.g. Thermo ExactFinder) and informatics tools (e.g. spectral library searching, literature data) for the identification of emerging environmental contaminants in the aquatic environment *without* the aid of user defined molecular databases. In this approach, high resolution ( $R > 100,000$ ) accurate mass (mass error  $< 2$  ppm) chromatograms of wastewater extracts are subjected to recursive peak assignment, adduct grouping, isotope pattern scoring, and molecular formula assignment. Chromatographic features and molecular formulas are culled using empirical indices (e.g., chromatographic peak shape, mass accuracy, isotopic pattern fit) and heuristic rules for the assignment of probable molecular formulas (e.g., Seven Golden Rules). Filtered chromatographic features are subjected to confirmation criteria for tentative identification. To this end, we demonstrate the capabilities of rule based *in silico* fragmentation and hydrogen/deuterium exchange experiments to aid in the structural elucidation of unknown contaminants without the benefit of reference standards. We have utilized this approach to identify novel organic microcontaminants in wastewater and wastewater-impacted surface waters. Results illustrate our ability to identify a broad range of organic contaminants in water, including several compounds that have not been previously reported to occur in environmental systems.

**590 Bridging environmental mixtures and toxic effects** S.E. Allan, / Environmental and Molecular Toxicology, Oregon State University / Graduate Researcher; B. Smith, Oregon State University / Environmental and Molecular Toxicology; R.L. Tanguay, Oregon State University / Environmental & Molecular Toxicology, Oregon State University / Department of Environmental & Molecular Toxicology; K.A. Anderson, Oregon State University / Environmental & Molecular Toxicology, Oregon State University / Dept. of Environmental & Molecular Toxicology. BRIDGES is a bioanalytical tool that combines passive sampling with the embryonic zebrafish developmental toxicity bioassay to provide a quantitative measure of the toxicity of bioavailable complex mixtures. Passive sampling devices (PSDs), which sequester and concentrate bioavailable organic contaminants from the environment, were deployed in the Willamette and Columbia Rivers within and outside of the Portland Harbor Superfund site in Portland Oregon. Six sampling events were conducted in the summer and fall of 2009 and 2010. PSD extracts were analyzed for PAH compounds and screened for 1,200 chemicals of concern using deconvolution reporting software. The developmental toxicity of the extracts was analyzed using the embryonic zebrafish bioassay. Significant spatial and temporal differences in the concentration of contaminants at the sites were observed. Similarly, significant differences in the developmental toxicity of the samples were recorded. This demonstrates the importance of utilizing an environmental monitoring tool, such as BRIDGES, that can provide site-specific, temporally resolved information about environmental contaminants and directly link environmental samples to toxicity. Multivariate modeling approaches were applied to paired chemical-toxic effects data sets to help unravel chemistry-toxicity associations. Although this research highlights the complexity of discerning specific bioactive compounds in complex mixtures, it demonstrates methods for associating toxic effects with chemical characteristics of environmental samples.

**591 Formation of Disinfection By-Products in a Drinking Water Treatment Plant: Bioanalytical and chemical tools provide complementary information** B.I. Escher, Entox, The University of Queensland / National Research Centre for Environmental Toxicology; P. Neale, Entox, The University of Queensland; A. Antony, The University of New South Wales / UNESCO Centre for Membrane Science and Technology; M.E. Bartkow, Seqwater / Queensland Bulk Water Supply Authority (trading as Seqwater); M. Farre, AWMC, The University of Queensland / Advanced Water Management Centre; A. Heitz, I. Kristiana, Curtin Water Quality Research Centre, Curtin University; J.Y. Tang, Entox, The University of Queensland / National Research Centre for Environmental Toxicology. Disinfection of drinking water is the most successful measure to reduce water-borne diseases and protect health. However, disinfection by-products (DBPs) formed from natural organic matter and disinfectants like chlorine and monochloramine may cause bladder cancer and other adverse health effects. Despite epidemiological evidence and identification of more than 600 individual DBPs, not all of the causative agents have been identified. We report on a pilot study that



uses bioanalytical tools to evaluate the water quality and the formation of DBPs through a full-scale water treatment plant serving a metropolitan area, which employs coagulation and sand filtration, followed by chlorination and chloramination. Water quality changes through the treatment process were investigated using *in vitro* bioassays (focusing on nonspecific and reactive toxicity), quantification of adsorbable organic halogens (AOX), characterization of organic matter as well as analytical quantification of selected regulated and emerging DBPs. Non-specific toxicity peaked mid-way in samples after the chlorination and storage steps. The general trend of all the quantified DBPs followed a pattern similar to the one seen for AOX with maximum concentrations observed in the final treated water. The mostly chlorinated and brominated DBPs formed during treatment also caused reactive toxicity to increase throughout treatment. Both genotoxicity with and without metabolic activation showed the same pattern as the non-specific toxicity with a maximum activity mid-way during the treatment train. The *E. coli* biosensors strains indicative of protein damage and glutathione depletion responded strongly to the chlorinated samples, while a bioassay indicative of the oxidative stress response did show some, albeit very small, activity. Despite the fact that bioassays were not optimized yet for volatile DBPs, the bioassays indicated an increase in toxicity associated with the formation of DBPs at the various stages of the treatment chain in this drinking water plant. It must be noted, though that cell-based bioassays are early warning indicators of an onset of effect or of repair and defense mechanisms. Thus their effect cannot be directly translated to an adverse health outcome but rather to the presence of groups of chemicals that exhibit reactive modes of toxic action.

**592 Monitoring Cap Effectiveness Using Bulk Sediment and Passive Porewater Sampling at the Wyckoff/Eagle Harbor East Harbor Superfund Site** D.D. Reible, The University of Texas-Austin, University of Texas / Environmental and Water Resources, The University of Texas at Austin / Dept. of Civil & Environmental Eng.; C.L. Thomas, The University of Texas-Austin / Civil, Architectural and Environmental Engineering, The University of Texas at Austin; X. Lu, The University of Texas-Austin / Civil, Architectural and Environmental Engineering; M. Michalsen, U.S. Army Corps of Engineers / Environmental Engineering & Technology Section; J. Wakeman, US Army Corps of Engineers / Biologist/Risk Assessor; T. Thompson, SEE, LLC. A 3-5 ft cap of clean sediment and borrow material was used to isolate PAHs introduced by a former creosote wood-treatment plant located on the east side of Bainbridge Island, Washington. As part of long-term monitoring, sediment cores and passive samplers were used in 2011 to measure bulk sediment and porewater PAH concentrations. Porewater concentrations were determined using solid phase microextraction (SPME) profilers with polydimethylsiloxane (PDMS) as the receiving phase sorbent. After review of the cores' bulk sediment PAH concentrations, SPME profilers were placed in areas where cap performance was suspect. Results were corrected for non-equilibrium conditions at the time of extraction by using deuterated PAHs to estimate absorption kinetics. Equilibrium correction factors ranged from 0.36 (dibenz[a,h]anthracene) to 0.91 (naphthalene). Corrected porewater and surface water (SW) SPME results were compared to surface water quality standards (SWQS). No near-surface intervals exceeded SWQS. Only one deep interval, at a depth of 33 in., exceeded the SWQS for benzo[a]pyrene. Three SW locations were examined to determine if levels near the cap compared to background levels. Depth averaged SW concentrations for one of two cap SW locations was greater than the background SW level by ~20%. No SWQS exceedances were noted at SW locations. Thus, the cap is currently effective at preventing recontamination of overlying water from deeper contaminated sediment. Observed porewater profiles showed no evidence of contaminant migration except in two anomalous locations. One location showed higher concentrations of LPAHs in the near surface suggesting a recent exposure to source-like material. Another location showed evidence of migration from below into the lower portions of the cap. Where SPME profilers and sediment cores were co-located, the profiler's upper 10 cm were compared to a porewater concentration converted from the core's bulk sediment concentration by equilibrium partitioning (EqP). Approximately 65% of the EqP predictions exceeded SPME measures by an order of magnitude, and about 5% exceeded by two orders of magnitude. This is commonly observed and likely attributable to the apparent reduced availability of the contaminants in the bulk solids due to stronger sorption to hard or black carbon phases. The results suggest that the direct measurement of porewater provides a better indication of contaminant migration and availability than bulk solid measures.

**593 Monitoring exposure of brown bullheads and macroinvertebrates to contaminated sediments in the Ashtabula River before, during and after dredging** J.M. Lazorchak, U.S. EPA, US Environmental Protection Agency / Office of Research and Development, U.S. EPA Office of Research and Development / National Exposure Research Laboratory, U.S. EPA / Molecular Indicators Research Branch, U.S. EPA / Molecular Ecology Research Branch; J. Meier, P. Wernsing, U.S. Environmental Protection Agency / NERL, Ecological Exposure Research Division; M.A. Mills, U.S. EPA, U.S. EPA / National Risk Management Research Laboratory, ORD, US EPA / Remediation and Pollution Control Division, NRMRL, U.S. EPA / Office of Research & Development; P. Baumann, Retired USGS. The Ashtabula River in Northeastern, Ohio has been designated by the International Joint Commission as a Great Lakes Area of Concern ([www.epa.gov/glnpo/aoc](http://www.epa.gov/glnpo/aoc)), based on the listing of six beneficial use impairments (out of 14 possible) in the lower two miles of the river. In 2007, approximately 550,000 cubic yards of contaminated sediments were removed through remedial dredging. The primary objective of this field investigation was to monitor contaminant exposure in selected biota before, during, and following dredging. This was done by measuring PCB and PAH accumulation in whole fish tissue (brown bullheads) and in benthic macroinvertebrates, assessing changes in DNA damage in liver and blood of the fish, and scoring external and histopathological lesions (including tumors) in the fish. Sediment and water samples were also collected in conjunction with the biological sampling to evaluate contaminant residuals. Levels of DNA damage in indigenous brown bullheads were measured using the Comet assay and compared to levels in fish collected at a reference site, Conneaut Creek. Benthic macroinvertebrates were collected using Hester-Dendy artificial substrates by deployment at three locations in the dredge area as well as at an upstream reference site. The pre- and during-dredging characterizations have been completed and four years of post-dredging data have been collected. Results thus far confirm a reduction in fish tissue and macroinvertebrate levels of PCB and PAHs following dredging, indicating the effectiveness of the remediation in reducing exposure of biota to the primary contaminants of concern. Similarly, although initially higher in fish collected pre- and during dredging, DNA damage levels in the Ashtabula fish post-dredging are similar to those seen in fish collected from the Conneaut reference site. When completed, the methods used in this study can be used by regulatory agencies, remedial project managers, and dredging practitioners to evaluate and assess the effectiveness of environmentally-prudent remediation techniques for contaminated sediment sites around the country. Although this work was reviewed by USEPA and approved for publication, it may not necessarily reflect Agency policy.

**594 Monitoring of Contaminated Sediments Before and After Dam Removal on 12 Mile River; Pickens County, SC** C. Zeller, US EPA – Region 4 / Environmental Engineer. In 1994, the Region 4 Office of the United States Environmental Protection Agency (EPA) issued a Monitored Natural Recovery (MNR) Record of Decision (ROD) for the Sangamo Weston/12 Mile River/Lake Hartwell Superfund Site located in Upstate South Carolina. Annual monitoring conducted since the ROD was issued indicates a steady decline of PCB concentrations in surface sediments of the 12 Mile Arm of Lake Hartwell that is attributed to physical processes such as burial, mixing, and dispersion. PCB concentrations in surface sediments have been below the 1 mg/kg cleanup level for the past several years. PCB levels in *corbicula* tissue have shown a 90% decline since source control efforts were largely completed in the mid-1990s, although additional studies have shown the need for further source control work. However, declines of PCB concentrations in fish tissue have not kept pace with those observed in surface sediments and *corbicula*. A Federal Consent Decree was issued in 2006 to settle Natural Resource Damage claims. A major component of the Trustee agreement involved the removal of two, turn of the century textile mill dams from 12 Mile River. This work was completed in 2011 and generally included hydraulic dredging/mechanical excavation of approximately 450,000 cubic yards (CYs) of sediment entrained behind the dams, sediment processing, water treatment, material disposal in a 10 acre Sediment Management Unit (SMU), and stream restoration. Concerns regarding PCB concentrations in residual sediments that remained in the 12 Mile River channel post dam removal has required EPA to initiate a Supplemental Remedial Investigation 18 years after the ROD was issued. This presentation will discuss the 12 Mile River dam removal project and how the monitoring program for the Sangamo Weston/12 Mile River/Lake Hartwell Superfund Site has been modified pre- and post-dam removal.

**595 Monitoring Sediment Cap Performance with Profiling SPME** D.D. Reible, The University of Texas-Austin, University of Texas / Environmental and Water Resources, The University of Texas at Austin / Dept. of Civil & Environmental Eng.; C.L. Thomas, The University of Texas-Austin / Civil, Architectural and Environmental Engineering, The University of Texas at Austin; X. Lu, The University of Texas-Austin / Civil, Architectural and Environmental Engineering; D. Lampert, Argonne National Laboratory. Sediment caps reduce the risk posed by the fate and transport of contaminants by stabilizing the underlying sediments, physically isolating the water column from sediment contaminants, and reducing contaminant flux to the benthic organisms and water column. Evaluating the performance of a placed cap is challenging, however, due to the long time frames associated with migration of hydrophobic contaminants through a cap. In addition, the non-sorbing nature of most sediment caps limits the usefulness of bulk solid phase measurements of contamination. Here, the freely dissolved contaminant concentrations can be measured using solid phase microextraction (SPME) profilers with polydimethylsiloxane (PDMS) as the receiving phase sorbent. The use of SPME profilers results in lower detection limits (ng/L) and in the ability to construct vertical concentration profiles that assist in the determination of the mechanisms and rates of transport within a sediment cap. This approach has been used at the University of Texas at a variety of contaminated sediments sites including the McCormick and Baxter Portland Harbor Site (Portland, OR), Pacific Sound Resources (Seattle, WA), Tennessee Products (Chattanooga, TN), Wyckoff/Eagle Harbor (Seattle, WA), San Jacinto River Waste Pits (Baytown, TX), and Roxana Marsh (Roxana Marsh, IN). Contaminants of concern at these locations include the 16 polycyclic aromatic hydrocarbons (PAHs) on the EPA's Priority Pollutant List, dioxins, and furans. This presentation will summarize the implementation of the approach and results at these contaminated sediment sites to illustrate the utility and usefulness of the approach. Approaches to evaluate kinetics of uptake onto the sorbent fibers by extrapolation from a few performance reference compounds will be presented. Results illustrating the nature and extent of contaminant mobility, intermixing during cap placement and recontamination at these sites will be summarized.

**596 Remediation and Monitoring of PCBs in the Upper Hudson River: Observations and Successes of the Second Year of Operations** E.A. Garvey, Louis Berger Group, The Louis Berger Group, Inc. / Site Assessment and Remediation; S. Gbondo-Tugbawa, J. Atmadja, The Louis Berger Group, Inc.; G. Klawinski, D. King, B. Conetta, US EPA Region 2. The 2002 Record of Decision for the Hudson River PCBs Site called for environmental dredging of approximately 2.65 million cubic yards of PCB-contaminated sediment in two phases. As part of the implementation of the remedy, performance standards for resuspension, residuals, and productivity were developed to promote accountability and ensure that the cleanup meets the human health and environmental protection objectives. Phase 1 of the remediation was conducted by General Electric from May 15 to December 13, 2009, including the removal of 280,000 cy of sediment and an extensive monitoring program. Based on the observations and lessons of Phase 1, as well as the input of a peer review panel, USEPA revised the performance standards and the monitoring requirements for Phase 2, which GE signed on to in December of 2010. GE began Phase 2 of the remediation in May of 2011. As a result of the revisions to the performance standards, GE made a number of modifications to sequencing and operation of the dredges. At the same time at the direction of the USEPA, GE also conducted extensive monitoring of water column PCB levels to document the transport of PCBs away from the various dredging operations. By the close of the dredging season in November 2011, GE had succeeded in removing more than 360,000 cy of sediment and 27,000 kg of PCBs. At the same time, GE also succeeded in remaining below the performance standard for PCB resuspension for the entire season, based on both water column concentrations and dredging-related PCB loads. This presentation will highlight the important successes of the Phase 2 operation as well as the important modifications to the dredging operations that led to the successful second year of dredging. Among the more important improvements believed responsible for the success was the requirement to target the depth of removal slightly below (about 6 inches) the estimated depth of contamination. Additionally, the operations were conducted so as to minimize the time from the start of a dredging area to its closure so as to minimize the resuspension of disturbed sediments. To facilitate these improvements, the performance standard for residual sediment contamination was streamlined so as to permit a rapid assessment of each dredging pass. The end result of the various improvements

was to yield dredging losses at the compliance monitoring locations that were less than 1% of the mass removed, more than a 50% improvement over the Phase 1 operation.

**597 Selenium Management at Cane Ridge Unit of the Patoka River National Wildlife Refuge and Management Area 2007 – 2011** D.W. Sparks, U.S. Fish & Wildlife Service. Cane Ridge management unit was created in 2004 to provide an alternate nesting site for the federally endangered interior least tern (*Sterna antillarum*) that been nesting on Duke Energy's Gibson Generating Station (GGS) located adjacent to the Wabash River, Gibson Co., Indiana. GGS consists of 5 coal-fired steam generating units (650 megawatts each) and a 3,000 ac man-made industrial surface impoundment used for providing cooling water to the generating units. Least terns first began nesting on GGS cooling pond center dike in 1986 and on the ash ponds, dredge flats, landfill and facility roads in the past decade. Duke Energy entered into a Habitat Conservation Plan to protect and manage interior least terns at the GGS. Cane Ridge is located immediately southwest of the cooling pond and water for Cane Ridge was supplied via gravity flow directly from GGS cooling pond from 2005 to May 28, 2008. Cane Ridge consists of the Tern Unit (TU) (59 ac), 4 moist soil units (25 – 68 ac), and bottomland forest. The TU includes two nesting islands that least terns have used since 2005. Duke Energy did not reopen Gibson Lake (cooling pond) for fishing on April 1, 2007 due to the presence of elevated levels of selenium (Se) in fish. Later in 2007, Duke Energy began investigating the extent of the Se problem in water and biota samples from all over the GGS and Cane Ridge. Se concentrations in water entering the tern unit from GGS cooling pond ranged from 11 to 14 ppb. TU fish had 14 – 40 ppm Se. Invertebrates in the TU (8 – 50 ppm Se) and moist soil unit C (1.9 – 11 ppm Se) posed an unacceptable risk to birds. The decisive action taken in May of 2008 to prevent further Se contamination of Cane Ridge was the proper decision. Several management actions were taken to reduce risk and exposures. In a little more than 2 years, almost every indicator shows that Se concentrations have significantly declined and are nearing or below concern levels. We appreciate Duke Energy's partnership in this process, and especially for their willingness to provide (at significant cost) a clean source of Wabash River water. It is disconcerting to realize that in just a few years (2005 – 2008), a Se problem could form in the shallow water wetlands created at Cane Ridge. What is worse, and even harder to admit, is that we should have predicted this.

**598 Source identification and tracking using multiple lines of evidence** M.A. Mills, U.S. EPA, U.S. EPA / National Risk Management Research Laboratory, ORD, US EPA / Remediation and Pollution Control Division, NRMRL, U.S. EPA / Office of Research & Development; D. Walters, USGS / Fort Collins Science Center, USGS / Ecologist; K. Fritz, USEPA; A.P. Mucha, U.S. Environmental Protection Agency / Great Lakes National Program Office; S. Cieniawski, U.S. Environmental Protection Agency / Great Lakes National Program Office; C. Zeller, US EPA – Region 4 / Environmental Engineer; J.M. Lazorchak, U.S. EPA, US Environmental Protection Agency / Office of Research and Development, U.S. EPA Office of Research and Development / National Exposure Research Laboratory, U.S. EPA / Molecular Indicators Research Branch, U.S. EPA / Molecular Ecology Research Branch. A primary tenet in the remediation of contaminated sediments is to assure adequate source control to avoid recontamination of the project area once remediation is complete. However, often the full extent of all sources is unknown or poorly characterized. Methods and approaches to identify and track sources are needed to locate contaminant sources and develop a database of the "signature or fingerprint" of sources contributing to a project area. Source identification and tracking has two primary purposes: 1) identify the sources of contamination and 2) to track and apportion the contribution of multiple sources if possible. Commonly, a historic legacy of contamination in a river, port, or harbor is from multiple sources (historic discharges, landfill plumes, combined sewer overflows, industrial effluents, etc). Apportioning the contribution from multiple sources in these systems can be important in identify responsible parties and liabilities. During the site investigation/site characterization stages of a project, a PM identifies sources to establish source control. Subsequent to source control efforts, demonstrating source control may be required throughout the remediation phase and subsequently during long term recovery and monitoring. Case studies will be presented to demonstrate multiple methods and approaches to conducting source identification and tracking. Sites will include Great Lake Areas-of-Concern



and a Superfund site. The methods and approaches will provide a weight of evidence using biological, chemical, and physical lines of evidence. These methods include such techniques as passive samplers, biological monitoring (bivalves and other invertebrates), sediment traps, bioavailable fraction assessments, etc.

**599 Tree swallows: A tool to assess remedy effectiveness** T.W. Custer, U.S. Geological Survey / Upper Midwest Environmental Sciences Center, U.S. Geological Survey; C.M. Custer, U.S. Geological Survey / Upper Midwest Environmental Sciences Center, U.S. Geological Survey / Upper Midwest Environ. Sciences Center; P.M. Dummer, U.S. Geological Survey. Even though a large investment of resources is often required to clean up contaminated sediment, assessment of how effective these activities are in lowering contaminant residues in resident fish and wildlife is often lacking. One approach to evaluate the effectiveness of sediment removal is to compare contaminant concentrations in bird tissues before, during, and after clean up. Tree swallows are an ideal species for this evaluation. They feed on emergent aquatic insects and concentrations in their eggs and nestlings reflect local sediment contamination. They nest in boxes and can be enticed to nest in a wide variety of aquatic habitats including industrial locations. They also integrate contamination over an appropriate temporal and spatial scale for this type of assessment. Contaminant concentrations in eggs and nestlings and accumulation rates (mass of a contaminant accumulated per day) in swallow nestlings can be compared before and after remediation. For example, mean polychlorinated biphenyl concentrations in tree swallow eggs from lower Green Bay, WI were statistically lower in 2010 (1.7 ppm wet weight) than in 1995 (3.9 ppm wet weight). As part of the Great Lakes Restoration Initiative, contaminant concentrations in tree swallow eggs and nestlings have been collected since 2010 at several sites in the Great Lakes that have or will undergo contaminated sediment removal. We will present an overview of the status of those investigations.

**600 A Computational Model Linking Oocyte Growth and Spawning to the Hypothalamic-Pituitary-Gonadal Axis in Fathead Minnow (*Pimephales promelas*)** K. Watanabe-Sailor, Oregon Health & Science University, Oregon Health & Science University / Environmental and Biomolecular Systems; M. Mayo, US Army ERDC; E.J. Perkins, US Army Engineering Research & Development / Environmental Laboratory, US Army ERDC / Environmental Processes & Effect Division, US Army Engineering Research & Development / Environmental Genomics and Genetics Team; D.L. Villeneuve, U.S. EPA / National Health and Environmental Effects Research Laboratory, U.S. EPA; G.T. Ankley, US EPA / Office of Research and Development, National Health and Environmental Effects Research Laboratory, Mid-Continent Ecology Division, U.S. EPA / National Health and Environmental Effects Research Laboratory. Reproduction is vital to the survival of all living organisms, and reproductive toxicity is an important outcome in determining the ecological risks of chemicals in the environment. To evaluate reproductive toxicity, fathead minnow fecundity, as measured by the average number of eggs spawned, is used as an endpoint. In order to predict the effect of impaired reproduction upon a population, population dynamic models use fecundity as an input. However, when experimental data are not available, there are only a few computational models that will predict fecundity, and they rely primarily upon plasma vitellogenin, a precursor to an egg yolk protein, as an input. These models employ a statistical correlation between plasma vitellogenin concentrations in females and cumulative egg production, or they simulate oocyte growth and spawning based upon the absorption of vitellogenin into oocytes and known statistical distributions for clutch sizes and spawning intervals. However, a detailed physiological understanding of oocyte growth and development exists and could be leveraged to create a more mechanistic computational simulation model for predicting fecundity. Such a model could potentially make use of an increasing amount and diversity of in vitro data that may emanate from a growing emphasis on predictive approaches to toxicity testing in order to extrapolate these data to predicted effects at the individual or population-level. Thus, there is a need to improve existing models of oocyte growth and spawning to better represent biological processes that may be affected by chemicals in the environment, and to utilize a wider variety of data. Toward this end, this presentation will describe the development of a Matlab®-based oocyte growth dynamics model linked to an HPG axis model for fathead minnow (*Pimephales promelas*). It extends a previously developed model for oocyte growth dynamics and links to a model of

the hypothalamic-pituitary-gonadal axis through plasma vitellogenin and selected steroid hormones.

**601 How to Improve Prediction of Contaminant Bioaccumulation by Taking into Account Lag Time: an Example of Selenium in a Flowing System** W. Beckon, U.S. Fish and Wildlife Services / Division of Environmental Contaminants. For bioaccumulative contaminants, such as selenium, efforts to predict concentrations in organisms at upper trophic levels, based on measurements of environmental exposure, are confounded by several factors. Principal among these factors is the appreciable amount of time it may take for bioaccumulation to occur through various pathways and across several trophic transfers. Yet studies of bioaccumulation have hitherto generally ignored this factor, using temporally matched samples of water and tissue, effectively making the unrealistic assumption that bioaccumulation is instantaneous. The study summarized here offers a remedy for this deficiency, demonstrating a simple, objective method of estimating the time required for bioaccumulation, and taking this lag into account to improve predictive modeling of bioaccumulation. Without the need for special tracers or isotope measurements, this method uses a history of conventional measurements of environmental exposure and tissue concentrations of a contaminant to systematically test a large array of potential lag characteristics (both central tendency and spread of lag), selecting the lag that provides the best regression. The method is demonstrated here using an unusually long-term data set of selenium measurements in water and aquatic organisms in sloughs in the San Joaquin Valley in California. Preliminary results of this application of the method indicate that, as expected, more time is required for selenium to bioaccumulate in organisms at higher trophic levels than in those at lower trophic levels. For example, the lag time for the piscivorous largemouth bass (*Micropterus salmoides*) may be more than one year, but only about one month for the filter-feeding threadfin shad (*Dorosoma petenense*). Also as expected, omnivorous organisms display a broader spread in lag time than more specialized feeders. Therefore, the method presented here not only provides a tool for improved prediction of contaminant effects and for more precise targeting of contaminant regulation, it also offers a novel means to estimate both the average trophic level of an organism and its trophic spread (degree of omnivory), when these characteristics are otherwise poorly known. More generally, this method may improve the precision of predictive modeling in a wide variety of cause-effect relationships in which lag time is substantial but inadequately known, in disciplines as diverse as climatology and economics.

**602 Investigation of microfluidic cultures for the assessment of endocrine disruption in fathead minnows** T. Kissane, University of Illinois; D. Crokek, R. Wilson, US Army Corps of Engineers ERDC-CERL. *In vitro* studies on endocrine disrupting compounds (EDCs) commonly focus on individual cell or tissue types. While providing useful information, these systems do not include the impact of other endocrine tissues upon the investigated tissue. Numerous components of the endocrine system were cultured within a microfluidic device, allowing hormone communication among all components. A device was designed to culture the brain (hypothalamus), pituitary, testes, and liver (HPG-L) of fathead minnow (*Pimephales promelas*). By altering flow of media and arrangement of the tissue cultures, the device allows manipulation of tissue hormone communication. Individual tissues can be removed to determine their influence upon endocrine disruption of individual chemicals, while changes in position or flow allow manipulation of when each tissue is exposed to the media and when that tissue alters the media relative to the other tissues in the system. Techniques were designed such that device assembly does not damage tissue viability. Tissues remain viable for 3 days or longer permitting long-term exposure of tissue. Media can be analyzed for key hormones including 11-ketotestosterone and vitellogenin, which are important indicator hormones for endocrine disruption. Future studies can include removal of tissue and determination of changes in protein expression. This device can serve not only as a method of assessing presence of EDCs from a source, but can aid in gaining a better understanding of the HPG-L axis feedback mechanisms.

**603 Population genome scans reveal structural variation that alters gene expression, individual fitness and population-level response to metals** J.R. Shaw, Dartmouth College / The School of Public and Environmental Affairs and The Center for Genomics and Bioinformatics; E. Turner, R.N. Keith, Z. Smith, S.P. Glaholt, S. Paland, Indiana University / The School of Public and Environmental Affairs and The Center for Genomics



and Bioinformatics; J.K. Colbourne, Indiana University / The Center for Genomics and Bioinformatics. *Daphnia*, or the water flea, is a sentinel species of freshwater ecosystems. Their populations are defined by the boundaries of ponds and lakes, are sensitive to modern toxicants in the environment, and thus are used to assess the ecological impact of environmental change. Their short generation time, large brood sizes, and ease of laboratory and field manipulation have assured *Daphnia's* importance for setting regulatory standards by environmental protection agencies, testing chemical safety, monitoring water quality, and as a model for environmental genomics research. A hallmark of the genome sequence is a large number of duplicated genes that are most responsive to ecological challenges and are specific to the *Daphnia* lineage. In this study, we take advantage of maturing genomics tools to understand the molecular basis for evolved tolerance to toxic levels of certain metals. We also test the adaptive significance of *Daphnia's* genome structure. Natural populations that have faced severe chemical challenges for over a century of industrial iron/ore smelting demonstrate evolved tolerance to cadmium. Other reference populations that have no history of chemical stress are clearly harmed by metal exposure, showing slower growth rates, lower fecundity and higher mortality. By measuring the distribution of copy number variants (CNV) and interrogating differential expression of 31,000 annotated genes from sampled populations across chemical conditions, this study provides new insights into the functional interactions between genome structure and environment. We identify a large number of CNV (13.5%), including the metal detoxication protein metallothionein that strongly correlate and are predictive of phenotypic differences between populations. Dynamic networks constructed from these CNV reveal functional relationships between genes that are consistent with mechanism of action. These studies begin to quantitatively link genomic variation with individual fitness and population-level outcomes, and both benefit from and contribute to the *Daphnia* Genomics Consortium.

**604 Quantifying the impact of pesticides on interspecific competition: An application of Bayesian hierarchical modeling to ecotoxicology** R. Erickson, Texas Tech University / Dept. of Environmental Toxicology, Texas Tech University / Department of Environmental Toxicology, Texas Tech University / graduate student; J.L. Oates, T.A. Anderson, Texas Tech University / Dept. of Environmental Toxicology; K.R. Long, Texas Tech University / Dept. Of Mathematics; S.B. Cox, Research and Testing, LLC.. Ecotoxicology has been moving towards the use of population models to study toxicants. These models add realism by allowing for the inclusion of multiple ecological factors and the exploration of different exposure scenarios. Traditionally, these studies have been done with single species and do not explicitly consider interspecific interactions. We examined how different pesticides altered the population dynamics of two different species of *Daphnia*. We tested three hypotheses: 1) Pesticides would change interspecific competition, 2) Pesticides would change resource use and intraspecific competition, 3) Pesticides would change mortality and fecundity. We tested these hypotheses using a laboratory study with two species of *Daphnia*: *D. magna* and *D. pulex*. Populations of each species were raised in monocultures and bicultures. The system was independently exposed to a high and low dose of two different pesticides: malathion and pendimethalin. Doses were chosen based upon a dose response pilot study and were the LC10 and 10% of the LC50 for the more sensitive species, *D. pulex*. The populations were sampled throughout the experiment and these observations were to parameterize a stage-structured matrix population model. A Bayesian hierarchical approach was used for model parameterization. Both doses had minimal effect to the monocultures. Under control settings, *D. pulex* outcompeted *D. magna* in the bicultures. Pendimethalin exposure altered the interspecific competition ability of the species. These led to a change in outcome from the competition study. Malathion altered mortality and fecundity of the two species. However, this did not alter the outcome of the competition study. Our findings demonstrate that ecological effects such as interspecific competition may need to be considered in population models in ecotoxicology.

**605 Reverse engineering the molecular machinery underlying the response of fathead minnow (*Pimephales promelas*) to the endocrine disruptor ketoconazole** E.J. Perkins, US Army Engineering Research & Development / Environmental Laboratory, US Army ERDC / Environmental Processes & Effect Division, US Army Engineering Research & Development / Environmental Genomics and Genetics Team; X. Wang, T. Habib, T. Habib, Badger Technical Service; N. Garcia-Reyero, Mississippi State University / Institute for Genomics Biocomputing and Biotechnology;

D.R. Johnson, U.S. Army Engineer Research and Development Center / Environmental Laboratory, U.S. Army / CEERD-EP-R, US Army Engineer Research and Development Center / Environmental Laboratory, US Army Engineer Research & Development Center / Environmental Laboratory. The low levels of multiple chemicals present in the environment and a poor understanding of compensation mechanisms in fish complicate estimation of the risks posed by endocrine disrupting chemicals. We have applied a systems biology approach to understand effects of endocrine disrupting chemicals in the Hypothalamic-Pituitary-Gonadal axis in the fathead minnow (*Pimephales promelas*). Using network inference techniques we have identified potential pathways and transcriptional regulators underlying compensation responses to endocrine disrupting chemicals. We exposed fathead minnow ovaries in vitro to the fungicide ketoconazole for ten time points over 150 min. Dynamic Bayesian Network analysis of microarray data from these exposures to identified transcription factors and cofactors interacting with key genes involved in steroidogenesis. The transcription factor SF-1 and cofactor MIER-1 were identified as possibly regulating the low-density lipoprotein receptor gene, LDLR. We validated this interaction by co-transfection of a catfish ovary cell line with a zebrafish LDLR promoter-green fluorescent protein fusion with constructs over expressing zebrafish SF-1 or zebrafish MEIR-1 genes. Over expression of either gene resulted in suppression of green fluorescent protein expression indicating that both SF-1 and MEIR-1 can regulate LDLR supporting involvement of these genes in fish responses to endocrine disruptors. This work demonstrates that reverse engineering approaches can be used to identify new regulatory mechanisms underlying chemical responses in ecologically important species.

**606 The Use of Multiple “Omic” Platforms to Evaluate the Effects of Endocrine Disrupting Compounds in Small Fish Species** K.J. Ralston-Hooper, Visiting Dupont Research Scientist at Duke University / Nicholas School of the Environment, DuPont / Haskell Global Centers; G.J. Getzinger, Duke University / Nicholas School of the Environment; M.E. Turner, E.J. Soderblom, M.A. Moseley, Duke University School of Medicine / Proteomics Core Facility; B.L. Escalon, US Army Engineer Research and Development Center; N. Garcia-Reyero, Mississippi State University / Institute for Genomics Biocomputing and Biotechnology; C. LaLone, U.S. EPA; D.L. Villeneuve, U.S. EPA / National Health and Environmental Effects Research Laboratory; G.T. Ankley, US EPA / Office of Research and Development, National Health and Environmental Effects Research Laboratory, Mid-Continent Ecology Division; R. Hoke, DuPont, Haskell Global Centers, E.I. DuPont de Nemours and Co., Haskell Global Centers for Health and Environment; L. Ferguson, Duke University / Department of Civil and Environmental Engineering, Duke University / Nicholas School of the Environment, Pratt School of Engineering / Department of Civil & Environmental Engineering. Linking molecular changes at multiple levels of biological organization using “omic” methods provides highly complementary, “data-dense” information for predicting outcomes for organisms exposed to environmental contaminants. However, performing separate “omic” analyses on multiple organisms or tissues increases the variability in responses and the uncertainty of the linkages between these levels of organization. Ideally, performing transcriptomic, proteomic, and metabolomic analyses on the same sample of individual tissues of interest from an organism would reduce this uncertainty and biological variation. On-going research in our laboratory has demonstrated the ability to apply multiple “omic” technologies to analyze individual tissues of small fish species. In the present study, transcriptomic and proteomic analyses were performed on hepatic tissues from individual adult female fathead minnows (FHM; *Pimephales promelas*) exposed to the aromatase inhibitor fadrozole. Organisms were exposed to 0, 0.04, and 1.0 µg/L fadrozole for 4 days and tissue extracts were analyzed using an LC-MS/MS based, label-free proteomics approach that identified over 1000 proteins. Many of the differentially expressed proteins in fadrozole-exposed female livers were consistent with published results for fadrozole. Interpretation of the broader proteomic response will be discussed in the context of transcriptomic data collected using mRNA isolated from the same samples used in proteomic analyses. Our data demonstrates the ability to extract high quality RNA and protein samples from small quantities (10 mg) of single tissues for comprehensive ‘omic’ analyses. Overall, our ability to perform multiple “omic” analyses on individual tissues of small fish species provides greatly improved information for connecting various levels of biological organization and elucidating pathways of toxicity. This information provides a more comprehensive

representation of the “systems effects” resulting from chemical exposures and will ideally serve as a basis for advancements in predictive ecotoxicology.

#### 607 Towards a Systems Framework for Defining & Using Adverse Outcome Pathways

S. Edwards, U.S. Environmental Protection Agency, US EPA / Integrated Systems Toxicology Division; R.B. Conolly, U.S. EPA / Integrated Systems Toxicology Division; M. Breen, US EPA; N. Garcia-Reyero, E. Perkins, T. Habib, M. Mayo, US ACE; D. Villeneuve, G. Ankley, US EPA; K. Watanabe, Oregon Health & Science University. High throughput screening (HTS) approaches are increasingly used to estimate chemical toxicity, and so there is a critical need for a robust framework that links the perturbation of these toxicity pathways to population level changes for application in risk assessment. To address this need, the concept of an adverse outcome pathway (AOP) was proposed where the molecular initiating events assayed by HTS are linked to population changes via a series of intermediate events. An open question, however, is how to determine whether specific key events have been missed when defining an AOP. In addition, there is increasing pressure to consider the cumulative effects of chemicals on wildlife populations rather than those of individual chemicals. While the AOP concept can be used for chemicals with an overlapping mechanism, cross-talk among different AOPs is certain to occur and must be considered to fully account for cumulative effects when relying solely on HTS data. Finally, species differences in AOPs will be important given that a limited number of laboratory species/strains will be used to define and characterize each individual AOP. A systems model based on a data-derived transcriptional network from 1,472 ovary samples will be used to show how all of these issues can be addressed through judicious use of discovery experiments leveraging various Omics technologies in combination with targeted experiments to quantitatively define the relationships among key events. First, the data-derived network will be used to evaluate a previously defined conceptual model on the hypothalamus-pituitary-gonad (HPG) axis in *Pimephales promelas*. Second, AOPs will be defined in a network context by leveraging both the literature-based conceptual model and the data-driven network model to evaluate the potential for cross-talk. Third, the network will be used to prioritize key events from the AOPs for inclusion in a computational model of the HPG axis used for predicting the effects of environmental chemicals on fecundity in *Pimephales promelas*. [This is an abstract or a proposed presentation and does not necessarily reflect EPA policy.]

#### 608 Association of carcinogenic PAH Emissions with Lung Cancer Mortality Rates on a Global Scale

O. Motorykin, Oregon State University / Department of Chemistry; M. Matzke, Pacific Northwest National Laboratory / Computational Biology and Bioinformatics; S. Simonich, Oregon State University / Depts. of Chemistry and Environmental & Molecular Toxicology; K. Waters, Pacific Northwest National Laboratory / Computational Biology and Bioinformatics. Four out of five lung cancer cases have been linked to tobacco smoking. Other factors, including diabetes, obesity, and personal income, have been associated with lung cancer rates. Air pollution, containing carcinogenic polycyclic aromatic hydrocarbons (PAHs), may also contribute to lung cancer occurrence and mortality. However, a clearer understanding of the contribution of air pollution (and carcinogenic PAH exposure) to lung cancer occurrence and mortality is need for better risk characterization, especially on a global scale. The estimated number of lung cancer deaths per 100,000 people in 2004 and age standardized lung cancer death rate per 100,000 people in 2004 were regressed on smoking prevalence, cigarette price, GDP per capita, number of people with diabetes, average body mass index (BMI) and carcinogenic PAH emissions (in benzo[a]pyrene equivalence) using multiple linear regression and data from 136 different countries. The contribution of carcinogenic PAH emissions to lung cancer mortality rates was assessed for countries with different socioeconomic groupings. A statistically significant positive correlation ( $p < 0.05$ ) between the estimated number of lung cancer deaths per 100,000 people and benzo[a]pyrene equivalent emissions was found for the high income level group (countries with GDP per capita  $> \$10,065$  USD) and not for low ( $< \$825$  USD per capita), low middle ( $\$826$  to  $\$3,255$  USD per capita), and upper middle ( $\$3,256$  to  $\$10,065$  USD per capita) income level groups. For every 10% increase in the mean BaPeq emissions, a 0.80% increase in the median number of lung cancer deaths per 100,000 people was observed for the high income level group. Similarly, a 10% increase in smoking prevalence resulted in a 4.5% increase in the median number of lung cancer deaths per 100,000 people for the high income level group ( $p < 0.05$ ). There was a statistically significant positive correlation ( $p < 0.05$ ) between smoking

prevalence and lung cancer mortality rates when all income level groups were combined. There was a statistically significant negative correlation ( $p < 0.05$ ) between cigarette price and the age standardized lung cancer death rate per 100,000 people when all income level groups were combined. This study is the first to link PAH emissions with lung cancer on a global scale. Future assessment of lung cancer risks should take into account air pollution.

#### 610 Engagement of Native American Tribes in the characterization of novel PAH dietary exposure pathways, assessment of possible human health risks

N.D. Forsberg, Oregon State University / Environmental and Molecular Toxicology; D. Stone, A. Harding, Oregon State University; B. Harper, S. Harris, Confederated Tribes of the Umatilla Indian Reservation; M.M. Matzke, Pacific Northwest National Laboratory; A. Cardenas, Oregon State University; K.M. Waters, Pacific Northwest National Laboratory; K.A. Anderson, Oregon State University / Environmental & Molecular Toxicology, Oregon State University / Dept. of Environmental & Molecular Toxicology. Although it is known that legacy toxicants, such as polycyclic aromatic hydrocarbons (PAHs), can be introduced into meats via smoke curing, little is known about their prevalence in smoked salmon prepared using traditional Native American smoking techniques. This work sought to characterize the effect of traditional Native American fish smoking methods on dietary exposure to 33 legacy and emerging substituted PAHs and identify possible risks to human health. Salmon smoking events were carried out by Tribal researchers at the Confederated Tribes of the Umatilla Indian Reservation in collaboration with Oregon State University researchers. Fresh caught Chinook salmon were smoked using two commonly used smoking structures (tipi or shed) and two types of traditionally used woods (apple or alder). For the purposes of exposure pathway and risk assessment, all salmon samples were prepared as if to be eaten. Additionally, 20 non-smoked spring-run Chinook salmon were analyzed for background PAH content along with three commercially available smoked salmon. Salmon samples were subsequently analyzed for PAHs. Potential carcinogenic and non-carcinogenic risks to human health were evaluated using Relative Potency Factor and Hazard Index approaches. Across all smoking methods, individual PAH loads ranged between  $< 2 - 3800$   $\mu\text{g/kg}$ , where non-carcinogenic, carcinogenic, emerging, and legacy PAHs generally accounted for 95%, 5%, 20% and 80% of the total PAH load respectively. It was found that neither smoking structure nor wood type accounted for differences in smoked salmon PAH content. However, carcinogenic and non-carcinogenic PAH loads in traditionally smoked salmon were 40 – 430 times higher than PAH loads measured in fresh caught non-smoked salmon and commercial smoked salmon. Exposure to the levels of carcinogenic PAHs measured in traditionally prepared smoked salmon could result in excess life-time cancer risks between  $1\text{E-}5$  and  $1\text{E-}4$  at a daily consumption rate of 5 g/d and could approach  $1\text{E-}2$  at 300 g/d. Exposure to non-carcinogenic PAHs could result in hazard indexes of 0.005 at 5 g/d and approach 0.3 at 300 g/d. Levels of PAHs present in smoked salmon prepared using traditional Native American methods potentially pose elevated cancer risks if consumed at high consumption rates over many years. Study results were shared with the Tribes and are currently being evaluated in order to design a culturally specific Tribal message.

#### 611 Background Polycyclic Aromatic Hydrocarbon Concentrations in Soil

C. Liu, CDM Smith; T. Marcum, CDM Smith / Environmental Scientist; N. Luke, CDM, CDM Smith Inc.. Polycyclic aromatic hydrocarbons (PAHs) are formed through incomplete combustions of most organic material. PAHs are associated with many industrial and municipal activities as well as natural and anthropogenic sources – most notably, combustion of fossil fuels, including automobile and industrial emissions, structural fires, and various industrial activities form PAHs, as do natural occurrences, such as wild fires and volcanic activities. As a result of these many sources, PAHs are found in virtually all surface soils in both urban and rural areas. PAHs are commonly identified as risk drivers in environmental contamination cases because of their high cancer potencies and toxicity. The majority of the risk posed by PAHs is due to the seven carcinogenic PAHs (cPAHs) including benzo[a]pyrene. Risk-based cleanup levels for a residential scenario for cPAHs corresponding to a lifetime incremental cancer risk of one in a million to 10 in a million range from 0.015 to 0.15 mg/kg benzo[a]pyrene equivalents, less than typical background soil concentrations. The U.S. Environmental Protection Agency is proposing to expand cPAHs from seven to 24 compounds, which will result in even more restrictive cPAH cleanup levels. Given their ubiquitous presence in the environment, it is important to



compare risk-based cleanup levels with typical background levels. However, the availability of such data is limited. This paper compiles PAH background levels in soil using state established background concentrations and information from a variety of published studies. The data for PAHs could define the range of PAH concentrations typical of background, provide the scientific data needed for identifying PAH source, and provide the basis for amending the risk-based soil cleanup levels to account for background PAH occurrence.

**612 Critical Evaluation of USEPA's Toxicological Assessment of Benzo(a)pyrene** B.H. Magee, K. Connor, D. Chin, ARCADIS. USEPA's (EPA) draft Integrated Risk Information System (IRIS) Assessment of Benzo(a)pyrene (BaP) is due to be released for public review in June, 2012. The document will present a new Oral Slope Factor that will replace the current value. In addition, EPA will present four new values that are not currently on EPA's IRIS database: an Inhalation Unit Risk, a dermal Cancer Slope Factor, an oral Reference Dose, and an inhalation Reference Concentration. In advance of the document's release, the authors have performed a literature search to identify the available studies that could potentially meet EPA's study quality criteria as critical studies in the IRIS program. In addition, the authors have performed dose-response modeling using EPA's benchmark dose model to identify *point of departure* doses and composite uncertainty factors to derive the five toxicological reference values in accordance with standard EPA policy and guidance. The authors have also evaluated alternative approaches to characterize the uncertainties in deriving the above toxicological reference values. In so doing, the authors have identified the toxicological reference values likely to be presented in EPA's draft document. This research will be used to prepare detailed comments to the EPA docket on EPA's toxicological assessment of BaP. If the document is released as scheduled, the presentation will compare the authors' proposed toxicological reference values to the EPA's proposed values and critically evaluate the scientific merit of the EPA's toxicological assessment of BaP. Specifically, the presentation will summarize the comments prepared for and submitted to the EPA docket. Additionally, the presentation will discuss the implications for the regulated community of the proposed toxicological reference values coupled with the Relative Potency Factors (RPFs) that are due to be issued by EPA in final form in 2012. Updated whole mixture validation exercises of carcinogenic endpoints will also be presented.

**613 Can the wealth of toxicity data for PAHs be a 'goldmine' for drug discovery and/or safer chemicals?** N. Gathergood, Dublin City University / School of Chemical Sciences; T. Hayes, Dublin City University / School of Chemical Sciences; M. Pour, Charles University / Department of Inorganic and Organic Chemistry, Faculty of Pharmacy; T. Garcia, IQAC-CSIC / Department of Surfactant Technology. Our design philosophy is to refine existing models on toxicity and biodegradation of PAHs to aid in the design of safer compounds and to establish a structure toxicity relationship of oxygenated PAH organocatalysts (BINOLs), or ligands for organometallic catalysts. At the same time to combine environmental toxicology screening with medicinal chemistry screening (possible output is a new hit compound). This approach has been shown successful in discovering hit MRSA selective Ionic Liquid during a study to probe toxicity of aminoacid containing ILs. We argue that the search for a better performance catalyst involves modification of ligand/catalyst scaffold. Analogous to medicinal chemistry SAR study. We believe that improved catalyst design can be coupled to the search for (the design of) safer materials and compounds. Toxicity and biodegradation data for oxygenated PAH organocatalysts will assist in the design of sustainable, efficient catalysts for synthesis and the pharmaceutical industry. This approach is possible (and indeed complements) due to the availability of toxicity data of a wide range of PAH compounds. We have successfully identified a highly toxic (antimicrobial and cytotoxic) PAH catalyst, which fails a 'CO<sub>2</sub> HeadSpace' biodegradation test. If this compound persists in the environment this is a significant cause for concern. In addition, based on our results, guidelines on how to reduce toxicity of PAHs (BINOLs) will be presented as well as biodegradation data. Finally, our 'hit' selective antimicrobial drug PAH analogue will be disclosed, which was discovered due to the novel approach outlined above.

**614 Importance of Non-aqueous Hydrocarbon Phases while Evaluating the Toxicity of Polycyclic Aromatic Hydrocarbons in Contaminated Sediments** T.W. Valenti, Syngenta Crop Protection LLC / National Research Council, National Research Council / U.S. Environmental

Protection Agency; L. Heinis, U.S. EPA / MED-Duluth; T.L. Highland, U.S. EPA / ORD, NHEERL, Mid-Continent Ecology Division; J.R. Hockett, U.S. EPA / Mid-Continent Ecology Division; S. Ireland, U.S. EPA / Great Lakes National Program Office; C. Jenson, U.S. EPA; T.J. Norberg-King, U.S. EPA / ORD, NHEERL, Mid-Continent Ecology Division, U.S. EPA / Mid-Continent Ecology Division; D.R. Mount, US Environmental Protection Agency / ORD. Polycyclic aromatic hydrocarbons (PAH) are widely recognized as potential chemical stressors in aquatic environments and often occur as complex mixtures generally affiliated with oils, tars, or other non-aqueous phases. Recent laboratory experiments suggest that non-aqueous hydrocarbon phases like these can contribute to toxicity beyond that attributable to their PAH content alone, which may explain misalignments between predicted toxicity based on PAH concentrations and that measured in sediment toxicity tests. Using mineral oil as a surrogate for non-aqueous phases, we consistently observed toxic effects for *Hyalella azteca* and *Chironomus dilutus*, but little sensitivity of *Lumbriculus variegatus*. In addition, mineral oil toxicity was not affected by addition of activated carbon, thus suggesting that toxicity might be caused by physical contact. Subsequent experiments using reference sediments spiked with mineral oil and a mixture of PAHs showed that toxicity beyond that attributable to mineral oil alone occurred only when very high concentrations of PAHs were present. Ultimately, these findings strongly suggested that while all organisms were susceptible to PAH exposure, *H. azteca* and *C. dilutus* were far more sensitive to non-aqueous phases often associated with environmental PAHs. To compliment these efforts, we exposed 1<sup>st</sup> and 3<sup>rd</sup> instar *C. dilutus* for different durations of time in the laboratory to 1) reference sediments spiked with either mineral oil or a combination of mineral oil and PAH mixture, 2) sediments from PAH-contaminated sites. We conducted experiments with both life stages because previous studies have demonstrated that earlier instars can be markedly more sensitive to contaminant exposure. Oil and grease content of all samples were quantified gravimetrically via solvent extraction and contrasted toxicity measured in unmanipulated sediments or those treated with coconut charcoal to bind PAHs. Overall the study results demonstrate that the toxicity of PAH-contaminated sediments may be underestimated if toxicity associated with non-aqueous phases in field sediments is not considered. This abstract does not necessarily reflect EPA policy.

**615 Ecological Risk Assessment for Decision-making in Restoration of the Bolsa Chica Lowlands, California** H. Ohlendorf, CH2M Hill. An extensive environmental sampling program and ecological risk assessment were important contributors to decision-making for restoration of the Bolsa Chica Lowlands in Orange County, CA. The site is being restored from an operating oil field to more favorable wildlife habitat that includes a range of coastal tidal and non-tidal wetlands as mitigation for development of port facilities in nearby Los Angeles County. The site is large (about 485 hectares [1,200 acres]) and complex. Before restoration there were more than 430 active or abandoned oil wells, along with associated pipelines, former tank farms, waste sumps, roads, and other related facilities on the property. Storm drainage enters from nearby urbanized areas. A Bolsa Chica Technical Committee (which included several federal and state agencies) was formed to evaluate the risks and develop an approach to clean-up and restoration. The environmental sampling effort included characterization of known or suspected sources of contamination and also a stratified random approach to determine if contamination occurred away from those sources (e.g., from waste disposal in unidentified areas). Over 230 chemicals of potential concern were initially identified for the site. Our ecological risk assessment identified 70 chemicals that presented potential risks to plants or animals that are expected to use the site after restoration. Using a weight-of-evidence approach, the Technical Committee developed clean-up criteria that focused on 14 of the risk-driver chemicals. Inclusion/exclusion of chemicals for cleanup was based on correlation with other analytes, detection frequency, co-location of exceedances of criteria, availability of toxicity values, and best professional judgment. Clean-up levels were identified for material to be managed on-site and material to be hauled off-site. In 2004, the U.S. Fish and Wildlife Service initiated the first construction phase of the Bolsa Chica Restoration Project, which was completed in 2006. Major components included removal and clean-up of oil extraction facilities from part of the wetlands area; restoration of full tidal influence through a new ocean inlet, bridge construction, and excavation of a tidal basin; creation and enhancement of aquatic habitats and intertidal wetlands; creation of feeding and nesting areas for threatened and endangered birds; and preservation of non-tidal wetlands.



**616 Post-registration wildlife incident monitoring schemes can be used to evaluate management decisions for pesticides** P. Berny, J. Vein, D. Vey, Vetagro-sup, campus vétérinaire / Toxicology; A. Decors, ONCFS / SAGIR Network. The EU has re-evaluated all the existing pesticides and, as a result, only 300-400 active substances are now available instead of over 900 in the late 90s'. Among the reasons for banning pesticides, ecological effects, including non-target poisoning have been considered. The SAGIR network has been established in 1986 in order to monitor wildlife diseases in France. All toxicological analyses are conducted at the same laboratory. Cases were retrieved from the SAGIR database. Several pesticides were selected for investigation: strychnine, some cholinesterase inhibitors (aldicarb, carbofuran, furathion, mevinphos), and anticoagulant rodenticides. Information regarding species, circumstances of exposure were taken into consideration. Time-series analyses from 1995 until 2010 clearly indicate that banning may be effective if the products are actively eliminated and not available from other sources or countries. Strychnine, for instance, is still detected, although it was partially restricted in 1984 and completely banned in 2000. A surprising result of our analyses is the detection of shifts in pesticide poisoning cases with obvious "re-emerging" toxicants in the recent years, such as lindane and alpha-chloralose. These results will be compared with toxicological accidents observed in domestic animals with the same products. Trends are very similar, and even more pronounced in domestic species. A review of the circumstances of exposure clearly points out that pesticide poisoning is commonly associated with deliberate abuse and that the persisting chemicals, as well as the "re-emerging" ones are all mostly detected in illicit use against wildlife. Most species of concern are protected and/or endangered species. A special focus is made on anticoagulant rodenticides, which are widely used in field control of rodents in France. The appropriate use of these compounds can be monitored thanks to post-registration monitoring and this monitoring clearly pointed out the efficiency of management decisions.

**617 Selenium Exposure and Reproductive Health of Select Avian Species Associated with a Large Iron Mine in Marquette County, Michigan, USA** L. Kaulfersch, Michigan State University Wildlife Toxicology Laboratory, Michigan State University; M. Zwiernik, M. Nadeau, W. Folland, C. Grohman, C. Martz, Michigan State University Wildlife Toxicology Laboratory / Department of Animal Science. Selenium (Se) is a biologically essential mineral that has the potential to illicit teratogenicity in oviparous species when beneficial exposures are exceeded due to the maternal transfer of selenium to eggs. Se tends to bioaccumulate in lentic systems, resulting in increased exposures to organisms occupying upper trophic levels. Birds with a diet consisting of fish and aquatic invertebrates have an elevated potential for exposure due to their consumption of prey items that can sequester excess selenium. Birds that occupy ecosystems near Ishpeming, MI may be exposed to elevated levels of Se due to mine associated surface water discharges. Concentrations of selenium in some of these discharges have exceeded EPA criteria in the past. However, there is significant uncertainty as to whether these concentrations are great enough to result in adverse effects. Measurement endpoints associated with individual health and reproduction were quantified in avian species including: hooded mergansers, wood ducks, tree swallows, eastern bluebirds, black-capped chickadees, house wrens, Canada geese, mallards, eastern kingbirds, and savannah sparrows. Artificial nest boxes were placed both at mine influenced and non-mine influenced watersheds. Fresh eggs were randomly sampled from each active clutch and added eggs were salvaged. Embryos were examined for gross deformities and total Se concentrations were quantified. Elevated selenium levels that exceeded the 16 mg/kg dw TRV for teratogenicity were found in wood duck and hooded merganser eggs at four study areas. Hatching success differed for hooded mergansers between reference (81%) and study (91%) areas. Fledging success for eastern bluebirds varied from reference (100%) and study (86%) areas.

**618 Science tailored to the Natural Resource Damage Assessment and Restoration Program** N. Beyer, U.S. Geological Survey / Patuxent Wildlife Research Center; S. Finger, USGS/BRD/CERC, Geological Survey NRDAR Coordinator / Columbia Environmental Research Center. The U.S. Department of the Interior's Natural Resource Damage Assessment and Restoration Program is authorized under provisions of the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA), Clean Water Act (CWA), and the Oil Pollution Act (OPA) to restore natural resources that are injured, destroyed or lost as a result of releases of hazardous substances

or discharges of oil. The Program has restored thousands of acres of habitat across the United States and over 1500 miles of shoreline. Natural resources include water, air, soil and biological resources that are held in trust by federal, state or tribal entities. Whether NRDAR cases are resolved through cooperative settlements or by litigation, they rely on the application of principles of environmental toxicology; both the determination of injury and the quantification of the loss of natural resource services and associated damages are based on sound toxicological, ecological, and economic sciences. Determining injury is not solely a scientific endeavor, but must be done within the context of legal regulations and definitions. In practice, sometimes the questions that seem most straightforward are the most difficult for a scientist to answer. Although research conducted under the Restoration Program may increase our general scientific understanding of ecotoxicology, its primary purpose is to provide the data necessary to resolve the case. The research biologist contributes just part of the story, which intertwines with contributions from attorneys, economists, managers and scientists in other fields. This presentation will provide examples of how injuries to both migratory birds and to a forest were related to metal concentrations in soil or sediment, and how those concentrations, in turn, were used to quantify injury and plan restoration.

**619 Improving risk predictions through the integration of space and habitat suitability: an application of the spatially-explicit exposure model (SEEM)** M.S. Johnson, US Army, Public Health Command / Health Effects Research Program, US Army Institute of Public Health / Health Effects Research Program, Army Institute of Public Health / Health Effects Research Program, US Army Ctr for Health Promotion & Preventive Med. / Health Effects Research Program; M. Quinn, US Army Institute for Public Health; T. Wickwire, Exponent Inc.. Interactions of biological entities within the environment occur on spatial and temporal scales. Likewise, the spatial and temporal distributions of contamination within the environment affect the degree to which plants, animals, and humans are exposed and how they respond. These interactions can be complex, however, through the recent advances in geographical information systems (GIS) and other models that integrate spatial considerations, estimates of risk can be more accurately described. Moreover, presentation of contaminated sites on spatial scales allow for a clearer understanding of the problem. The present study describes how a simple three-part delineation of soil lead (Pb) concentrations and three part habitat delineation can provide more accurate risk predictions. Risk predictions were verified through a comparison with blood Pb data from songbirds and small mammals collected from two test sites and compared with a blood Pb toxicity reference value. Both spatial risk predictions and those derived using simple deterministic approaches were compared and evaluated. Positive and negative aspects of each will be described.

**620 Demystifying Toxic Reference Values for Bald Eagles in Assessment of Damages** W. Bowerman, University of Maryland / Department of Environmental Science & Technology; D. Best, U.S. Fish and Wildlife Service / Environmental Contaminants Program. Bald eagles (*Haliaeetus leucocephalus*) are tertiary predators in many polluted aquatic ecosystems in the United States. The effects of many environmental pollutants on reproductive and developmental health of eagles are used in the NRDA process to determine damages. The literature, however, is confusing when determining Toxic Reference Values, since endpoints are inconsistently applied. Reproductive health TRVs for individual compounds have been published that utilize 1.0, 0.7, and near 0 young per occupied nest as reproductive endpoints of effects. These endpoints are based on differing effect levels tied to Federal Endangered Species Act recovery goals, theoretical population stability levels, and total reproductive failure, respectively. TRVs are available for both unhatched eggs and blood plasma levels for individual environmental pollutants. To further confuse the issue, most TRVs are based on field data for reproduction correlated to environmental contaminants in field collected tissues. However, TRVs have been determined from effects observed during periods where eagle populations were declining or stable, but also during the recovery of these populations where near exponential population growth was occurring. No correction factors have been calculated to account for these differences in population dynamics, which can greatly impact the validity of TRVs. In addition, for some compounds, TRVs are based on laboratory concentrations in other avian species used to model bald eagles, which make poor laboratory animal models. This study will demystify the endpoints related to these TRVs and the related problems used to determine damages to this Federal Trustee Species.

**621 Homing pigeons as useful biomonitors for atmospheric environmental decisions** R. Halbrook, Southern Illinois University / Zoology, Cooperative Wildlife Research Laboratory, TG&G Wildlife Consulting/ Southern Illinois University, Emeritus / Cooperative Wildlife Research Laboratory; W. Liu, Laboratory for Earth Surface Processes / College of Urban and Environmental Sciences, Peking University; S. Tao, Peking University / Laboratory for Earth Surface Processes; J. Cizdziel, University of Mississippi / Department of Chemistry and Biochemistry. Atmospheric pollution is a global concern affecting animal and human health. Air monitoring can provide information regarding concentrations of hazardous chemicals in the air; however, biomonitoring provides data not available from air monitoring alone, and can provide governmental and regulatory agencies with useful information for making environmental decisions. Know age, location, and health history information facilitate the use of homing pigeons to evaluate air pollution among various urban areas and regions. Homing pigeons collected from Beijing, China and Manila, Philippines had similar gross lesions, including gray/black discolorations of the lungs and several cases of enlarged and irregular testes (tumors) compared to pigeons collected from Chengdu, China, and Midland, Texas and Los Angeles, California, USA. Concentrations of polycyclic aromatic hydrocarbons (PAHs) in lung and liver tissue from Beijing pigeons were greater than concentrations in Chengdu pigeons, and mercury (Hg) concentrations in lung and liver tissues were greater in pigeons from Beijing compared to those from Chengdu and the USA. Analyses of PAHs and Hg in tissues of pigeons from Manila, and PAHs in pigeons from the USA are ongoing. Our preliminary results suggest that homing pigeons are valuable biomonitors of atmospheric pollution providing regulators with specific location contaminant data useful in environmental management decisions. Available data suggest that atmospheric pollution in Beijing and Manila is adversely affecting the health of avian species in those urban areas, which suggest that local human populations may also be adversely affected.

**622 Effects of Embryonic Exposure to PCBs on heart development in avian laboratory species** T. Carro, University of Maryland / Animal Science; T. Diaz, R. Sclarsky, University of Maryland; K. Dean, University of Lethbridge / Neuroscience, University of Maryland / post-doctoral research fellow; L. Carpenter, University of Maryland; M. Ottinger, University of Maryland / Department of Animal and Avian Sciences. Although the manufacture of polychlorinated biphenyls (PCBs) in the United States ceased in the late 1970s, these compounds remain ubiquitous in the environment and are linked with adverse health effects, such as endocrine disruption, decreased reproductive fitness, reduced immune responsiveness, and inhibition of neurological function. There is some evidence that PCBs adversely impact the cardiovascular system and our previous studies showed embryonic exposure to a coplanar PCB congener in wild birds affected the development of the ventricular wall compact layer, a critical morphological region of the heart, at concentrations that did not impact hatch success. To study the effects of environmentally relevant PCB mixtures on heart development, Japanese quail (*Coturnix japonica*) and broiler chicken (*Gallus domesticus*) embryos were treated with graded doses of PCB mixtures. Both mixtures affected hatching success at higher concentrations. Chicken hatchlings dosed with a 58-congener PCB mixture produced a variety of cardiomyopathies including ventricular hypertrophy, ventricular hypoplasia, abnormal septation, and the most common cardiomyopathy identified: noncompaction of the ventricular wall. Analysis of the Japanese quail hatchling hearts is currently ongoing. The effects noted in these hatchling studies suggest that environmentally relevant PCB mixtures do adversely affect hatch success and cardiac morphology of hatchlings following exposure *in ovo*. The cardiomyopathies identified in PCB-treated chicken hatchlings are consistent with congenital cardiovascular malformations identified in humans, which increase the risk of cardiovascular dysfunction, myocardial infarction, and ultimately contribute to overall heart failure. The conclusions and opinions presented here are those of the authors, they do not represent the official position of any of the funding agencies, the Hudson River Trustees, or the United States.

**623 Effects of copper contamination on aquatic ecosystem health** S. Gardham, Macquarie University / Department of Environment and Geography; G. Hose, Macquarie University / Department of Biological Sciences; A. Chariton, CSIRO / Land and Water. Copper has deleterious effects on the biodiversity and functioning of aquatic ecosystems at elevated concentrations. Guidelines for metals like copper are often derived from whole sediment toxicity tests. Although useful, this approach has been criticized

as it is based on single or a few species and considers neither the biological interactions among species nor environmental factors. Outdoor mesocosm studies complement whole sediment toxicity tests by accounting for these untested factors. This research considered the effects of copper on aquatic ecosystems with twenty 1500-L outdoor mesocosms. Clean sediments were spiked to create a gradient of copper contamination. The Australia and New Zealand Guidelines for Fresh and Marine Water Quality – Interim Sediment Quality Guidelines (ISQGs) were used to define nominal treatment concentrations of 0 (control), 32, 65 (low ISQG), 270 (high ISQG) and 540 mg/kg. The partitioning of a metal between particulate and aqueous phases is one of the most important processes controlling bioavailability. Laboratory spiked-sediments commonly have abnormally high porewater metal concentrations, which could confound the interpretation of results. Consequently, Simpson *et al* (2004) developed methods to create spiked sediments with realistic sediment geochemistry. We upscaled these methods for spiking at the mesocosm level. The concentration of copper in the sediment, porewaters and overlying waters was monitored over time. The majority of copper partitioned to the particulate phase with low µg/L concentrations measured in the porewaters and overlying waters in all treatments. The mesocosms were opened to biotic colonization in November 2010 and the development of the macroinvertebrate community was monitored for 1.5 years. Preliminary data shows the benthic macroinvertebrate community composition was significantly affected at and above the nominal treatment of 65 mg/kg, the low ISQG. Microbial degradation rates were affected above 32 mg/kg. Effects on genetic diversity, population and sub-lethal end points are also being considered. Our results show that we created environmentally realistic partitioning between sediment, porewaters and overlying waters. Under these conditions, concentrations of particulate-bound copper at guideline levels affected both the structural and functional characteristics of the benthic communities within the freshwater mesocosms.

**624 Recovery of a mining-damaged stream ecosystem** C. Mebane, US Geological Survey / NOAA Fisheries Liaison, US Geological Survey/WRD / NOAA Fisheries Liaison. The environmental science literature is replete with case studies of stream ecosystems damaged by metals pollution, but fewer case studies are available with long-term data on the recovery from effects of pollution. Panther Creek, a tributary to the Salmon River in central Idaho, USA, is one such case with a 30-year period of record from about 1980 through 2010. Panther Creek suffered intensive damage from large-scale mining and milling operations in the Blackbird Mining District, primarily from the late 1940s to 1967. The mine drained to two tributaries of Panther Creek, Blackbird Creek and Big Deer Creek, contaminating the streams with copper (Cu), arsenic, and cobalt (Co), as well as altering the physical channel. From the 1960s through 1980s, no fish and few aquatic insects could be found in mine-affected sections of Panther Creek. By the early 1990s, Cu concentrations had attenuated to the point that some natural production of rainbow trout occurred. However, Chinook salmon and resident fish other than rainbow trout remained rare. Species richness of aquatic insects was about half that of reference sites, and mayflies were absent or rare. Concerted efforts to reduce mine contamination began in 1995, and by 2002 Cu and Co levels had been reduced by about 90%. As of 2010, resident fish populations in mining-influenced reaches appeared to have recovered, relative to reference reaches. Rainbow trout were early colonizers, quickly expanding their range as areas became habitable. Sculpin appeared slower to disperse and colonize; but they appeared to fully recover about four years after their first detections. Macroinvertebrate richness in Panther Creek sites downstream of Blackbird and Big Deer Creeks appears to have plateaued at about 70 to 90% of reference. Total abundances of mayflies in locations downstream of mine influences are as high as or higher than at reference locations. However, mayfly diversity remains lower than reference, and Heptageniids and Ephemerellids remain rare. These patterns raise questions of what it means for an ecosystem to be “recovered.” For example, if benthic macroinvertebrates provide a sufficient food base for fish populations, is the ecosystem sufficiently recovered? Or would all major ecosystem components need to be similar to reference?

**625 Context dependent ecotoxicological responses to metals and other stressors** W.H. Clements, Colorado State University / Fish, Wildlife and Conservation Biology, Colorado State University, Colorado State University / Fish, Wildlife and Conservation Biology; C. Hickey, Natl. Inst. of Water & Atmospheric Res. / NIWA Ecosystems; K. Kidd, Canadian Rivers Institute, University of New Brunswick / Biology, University of New Brunswick /

Biology department. One of the major goals of ecotoxicology is to identify patterns that describe population and community responses to contaminants. Our ability to predict these responses is greatest for communities that change consistently in response to a specific contaminant or class of contaminants. However, we know that regional variation in community composition, which may result from environmental, historical, biogeographical and/or climatic factors, complicates our ability to identify general patterns of responses and represents a significant challenge for ecological risk assessment. In situations where responses to contaminants are highly variable, an understanding of the mechanisms responsible for differences among locations has important practical applications. For example, aquatic toxicologists have known for many years that certain physicochemical features of aquatic ecosystems such as pH, water hardness and dissolved organic carbon (DOC) greatly influence the toxicity and bioavailability of contaminants. In contrast to our well-developed understanding of how abiotic factors influence toxicity, we know relatively little about variation in sensitivity among communities. In fact, much of this variation is treated as “noise” and considered an impediment to generalization and to our ability to predict ecological responses. In this paper we suggest that variation among communities and along environmental gradients represents an opportunity to identify ecological factors that influence contaminant fate and effects. We introduce the concept of context dependency, which refers to variation in ecological patterns and processes across environmental or spatiotemporal gradients. We propose that many responses of aquatic communities to contaminants are context dependent and vary along environmental gradients. Specifically, we hypothesize that many of the environmental factors which structure communities in aquatic ecosystems (e.g., food web structure, disturbance, primary productivity, exposure history) also determine their responses to contaminants. Our ability to predict the effects of contaminants in aquatic ecosystems could be significantly improved by recognizing that responses to stressors are often context dependent and by developing a mechanistic understanding of context dependency.

**626 Improving Our Understanding of Ecologically Significant Metal Effects in Aquatic Benthos** A. Burton, University of Michigan / School of Natural Resources & Environment and Cooperative Institute for Limnology & Ecosystem Research; D. Costello, University of Michigan / School of Natural Resources & Environment; K.W. Custer, Wright State University / Department of Earth and Environmental Sciences; M. Li, University of Michigan / School of Natural Resources and Environment, Harvard School of Public Health / Environmental Health; S. Aselage, University of Michigan. A recent review by Brix et al. (2011) concluded there were often discrepancies between laboratory and field studies that assessed divalent metal effects on aquatic insects. The greater sensitivity observed in the field was attributed to inadequate exposure periods in the lab and reductions in primary producer biomass that resulted in indirect effects on grazers. Compiling results from many mesocosm and *in situ* experiments, we find support for complex interactions evident at the community and ecosystem level that may lead to discrepancies between lab and field studies. Under field conditions, spiked metals rapidly shifted their dominant partitioning relationships, thus indicating that short-term static lab exposures may not simulate field exposure. Furthermore, variation in DOC, sediment heterogeneity, and light exposure can modify sediment bioavailability. Sediment metals reduced food (i.e., biofilm) quantity and quality, which in turn affected grazer uptake and effects. Synergistic effects were observed between common stressors (P and Cu) in biofilm-grazer relationships. Finally, toxic effects may be modified by the route of exposure that dominates. More research is needed to better define the importance of these interactions and whether they can be modeled to better predict their ecological significance.

**627 Unraveling the disconnect between laboratory and field responses of aquatic insects to trace metals** D.B. Buchwalter, North Carolina State University / Department of Environmental and Molecular Toxicology, North Carolina State University / Department of Environmental & Molecular Toxicology. Aquatic insects are notoriously unresponsive to metals at ecologically relevant concentrations in laboratory toxicity tests, yet many authors report profound responses of insect communities to metals in the field. There are several potential reasons for this important disconnect, which can be broadly subdivided into three categories – time, diet, and indirect effects. I will present dissolved cadmium and zinc kinetics data (influx and efflux) for several aquatic insect species, demonstrating that the times required for most species to reach steady state tissue concentrations far exceeds the

duration of most toxicity tests. For example, time required to reach steady state tissue Cd concentrations ranges between 28 days in the fast effluxing *Hydropsyche californica* to >5,000 days (well beyond the lifespan) in the slow effluxing *Rhythrogena morrisoni*. Among 19 other species, time required to reach steady state Cd concentrations ranged from 41–716 days (mean = 155). Further, traditional toxicity assays do not consider dietary exposures, yet most studies that compare the relative importance of dissolved vs. dietary exposures in aquatic insects show that diet tends to be the more important exposure pathway in terms of overall metal accumulation. While many assume that diet derived metals are potentially less toxic than aqueous metals, a recent study in our lab shows the contrary: Antioxidant enzyme activities were negatively affected by dietary Cd whereas dissolved Cd exposures did not affect enzyme activities. Finally, little is known about how exposure to metals affects the quality and biomass of primary producers. Periphyton is the base of the food web in many aquatic ecosystems, and is a major sink for metals. Together, these observations suggest that traditional testing paradigms are not adequate for understanding the risks of metals to aquatic insects.

**628 A bioavailability-based approach to modeling metal mixtures: Part 1. model framework and conceptual considerations** A.C. Ryan, HDR | HydroQual; R.C. Santore, HDR|HydroQual, Inc.; J. Meyer, Arcadis U.S., Inc., ARCADIS; J.F. Ranville, Colorado School of Mines / Chemistry and Geochemistry, Colorado School of Mines. Evaluation of mixture toxicity can be extremely complicated and conclusions regarding additivity, less-than-additivity, or more-than-additivity can be influenced by several factors. For example, assessing metal mixture toxicity on the basis of dissolved metal does not consider the effects of solution chemistry on concentration of bioavailable forms of metals. Further, it does not consider competitive effects that individual component metals may have on one another. Utilization of a tool such as the Biotic Ligand Model (BLM) does consider these effects, and therefore minimizes the potential for error in characterization of exposure. Another factor that is important to consider is the uncertainty associated with concentration-response relationships for the individual metals present in a mixture. It is widely known that repeated single-metal toxicity tests will not consistently produce the same median lethal concentration. Not surprisingly, this implies that there is variability in concentration-response relationships. Characterizing variability in the response curves allows for development of confidence bounds around a central concentration-response relationship. Confidence bounds for the single metal responses can be combined to produce confidence bounds around a predicted response in a metal mixture toxicity test. These confidence bounds can be informative when making conclusions regarding additivity of the components in the mixture. To predict overall responses (i.e., percent mortality) for exposure to metal mixtures, a multi-site BLM was developed. This formulation of the BLM is essentially a simple combination of single-metal BLMs that already exist. Each component in a metal mixture is toxicologically active at a specific biotic ligand, but allowed to bind at all biotic ligands. This accommodates apparent competitive effects between toxicologically active and toxicologically inactive metals at each of the biotic ligand sites. Importantly, this approach is backward-compatible with single-metal BLMs (i.e., application of the multi-metal BLM to a single-metal exposure is identical to application of the single-metal BLM). In this presentation, the conceptual framework for the multi-metal BLM will be described. A companion presentation will describe application to multi-metal laboratory toxicity tests and to toxicity tests conducted in field-collected sediments.

**629 A bioavailability-based approach to modeling metal mixtures: Part 2. model application** R.C. Santore, HDR|HydroQual, Inc.; A.C. Ryan, HDR | HydroQual; J. Meyer, Arcadis U.S., Inc., ARCADIS; J.F. Ranville, Colorado School of Mines / Chemistry and Geochemistry, Colorado School of Mines. A Biotic Ligand Model (BLM)-based metal mixtures model with the capability to predict percent mortality (or survival) was applied to a number of organisms exposed to a series of binary and ternary mixtures of copper, cadmium, and zinc in laboratory and field settings. The model uses a response-additive approach to combine effects from individual metals. The model also considers uncertainty in each individual metal, and combines uncertainty estimates from individual metals into an overall uncertainty estimate for the mixture, which is characterized as a response envelope. Typically, these response envelopes are wide due to the high variability in responses to single metals and because of the steepness of the single-metal response curves. Most of the data considered fell within the response



envelopes, which are roughly consistent with 95% confidence bounds around a central response prediction, indicating that measured responses were well approximated by simple response additivity of the individual metal responses. Some of the data suggest either more-than- or less-than-additive responses, but the majority of the data suggest that the assumption of simple additivity is adequate. Generally, the mixture model performs reasonably well with laboratory tests with *D. magna*, as it can predict the patterns of responses observed in the toxicity data. For example, the model can account for the apparent beneficial effect of copper and zinc on cadmium toxicity (i.e., cadmium toxicity can be decreased with sub-lethal concentrations of either copper or zinc). The model was also successful in predicting toxicity to *H. azteca* in field collected sediments. This bioavailability-based approach was superior to a simple dissolved metal approach, despite the fact that water chemistry conditions did not appreciably vary in these datasets. In addition, the bioavailability-based approach can account for some of the apparently more-than- or less-than-additive effects of mixtures through consideration of competitive interactions at biotic ligands or competition for binding with inorganic or organic ligands in solution.

**630 Do mechanistic and/or empirical criteria predict toxicity in field collected sediments? An analysis of existing data sets** D.M. Di Toro, University of Delaware / Dept. of Civil & Environmental Engineering. Two methodologies exist that relate chemical concentrations to toxicity in sediments. Mechanistic criteria utilize observed toxicity from water only experiments and models of sediment-pore water partitioning to compute sediment criteria via equilibrium partitioning. Empirical criteria utilize large field collected data sets and develop sediment chemical concentrations that predict the probability of sediment being toxic. This paper examines the ability of both these methods to predict observed amphipod toxicity using three large data sets (I) "NOAA" data from the Status and Trends program (II) The "EPA" data collected and analyzed in "Predicting Toxicity to Amphipods from Sediment Chemistry" by LJay Field, SB Norton, DD MacDonald, CG Severn, CG Ingersoll (III) the "SCCWRP" data from "Sediment Quality Assessment Tools" data base by SM Bay, DJ Greenstein, JA Ranasinghe, DW Diehl, AE Fetscher These are large collections of data with many observations of paired amphipod survival and chemistry: NOAA (2469), EPA (4166), SCCWRP (8979) that have each been analyzed using various methods. The question to be answered in this presentation is: what fraction of the observed toxicity can be predicted using both criteria. The data will be analyzed in two ways. The methodology used to produce the empirical logistic regression models to determine the probability of finding amphipod toxicity will be reviewed and applied to the three data sets. In particular the screening procedure and the resulting logistic models will be examined. For the mechanistic criteria, the question becomes, what fraction of the observed toxicity can be attributed to the modes of actions that are the basis of the mechanistic criteria: SEM-AVS for metals and the total PAH criteria. For the latter criteria, regression models will be employed to estimate the missing data (TOC, SEM, AVS) so that a larger fraction of the data can be examined. The focus of this presentation will be the reverse of the previous approaches that focus on predicting toxicity. Rather the question will be: what fraction of observed toxicity is not explained by either technique. Possible causes and future investigations will be suggested.

**631 Sustainable Water Use: Comparative Evaluation of Widely Acclaimed Water Risk Tools** S. Quinn, The Procter & Gamble Company / Environmental Stewardship Organization; S.D. Dyer, The Procter & Gamble Company / Central Product Safety, The Procter & Gamble Company / Miami Valley Innovation Center, The Procter & Gamble Company / Miami Valley Labs. Growing human populations and economies coupled with global climate change have caused increased pressures on natural resources, including water. The sustainability of growth will require an understanding of appropriate conservation and use of water resources. Given the availability of globally digitized data, it is no surprise that over the past few years, several water risk assessment tools have emerged as a way for water-using entities to examine and promote their water sustainability as well as provide investors with tools that can evaluate the sustainability of various product sectors, even companies. Four of these tools include: the World Business Council on Sustainable Development's Water Global Water Tool, the World Wildlife Fund's Water Risk Filter, the World Resources Institute's Aqueduct Water Risk Atlas and the Global Environment Management Initiative's Local Water Tool. Each tool is designed to receive input data and then provide a risk scorecard based on input and other region-specific data. This

presentation provides results from our evaluation of these four water risk tools. In our evaluation we used manufacturing site data to compare each tool's function and output. These comparisons allowed us to determine the aspects of each tool that could contribute to a holistic and practical water risk assessment. The presentation of our results and conclusions will shed light on the constantly developing methods of water risk assessments and highlight ways that our work could contribute to more meaningful indicators for Life Cycle Assessment modeling (e.g. LCIA).

**632 A Water Analysis of Irrigation Related to Corn-based Ethanol production in the US** J.C. Bare, US EPA / MS-466, US EPA. Since the Renewable Fuel Standard has encouraged the development of biofuels, the US has seen an increase in corn production for conversion to ethanol. In many of these agricultural regions, increased corn production is accompanied with increased irrigation. A water use analysis will be presented that includes one regional scenario analysis of 12 US Midwestern states which is responsible for 88% of the corn production. Additional modeling will be done in the future to expand this analysis projecting two possible future scenarios for land use in 2020. The ArcGIS modeling was conducted with a 30 meter x 30 meter grid defining land use / land cover. Water stress indices were used to provide additional insights into the scarcity of water at each 30 meter x 30 meter location.

**633 Development of an integrated land use indicator in life cycle impact assessment based on ecosystem services** V. Cao, CIRAI, Polytechnique Montréal / Department of Chemistry, CIRAI / Department of Chemical Engineering; M. Margni, CIRAI, Polytechnique Montréal / Department of Mathematical and Industrial Engineering; L. Deschenes, CIRAI, Polytechnique Montréal / Department of Chemistry. In life cycle impact assessment (LCIA), potential impacts due to land use are calculated considering the land quality loss  $\Delta Q$  over area and time. Up to very recently, potential impacts were solely related to the loss of terrestrial biodiversity, which is not representative of all potential impacts generated by the use of land, as described by the JRC. The UNEP/SETAC Life Cycle Initiative project expanded the assessment framework by addressing additional impact pathways and related indicators, namely: biotic production potential (BPP), erosion regulation potential (ERP), fresh water regulation potential (FWRP), (mechanical and physico-chemical) water purification potential (WPP) and carbon sequestration potential (CSP). These represent provision and regulation services, as defined in the Millennium Ecosystem Assessment. Although this novel LCIA framework is more comprehensive, this development adds sophistication and potentially reduces the capacity of LCA to simplify the decision making by providing seven midpoints for land use alone. This project aims to normalize each midpoint to monetary values through economic valuation. Then, these converted midpoints can be aggregated into one stand-alone indicator, representing potential impacts of the land use as loss of ecosystem services natural capital. BPP and CSP are estimated with respectively productivity loss and carbon social cost, while the remaining regulation services are estimated with costs of technology compensation, as they are considered essential. As a local impact category, spatial variability and availability of the compensation systems are taken into account. Uncertainties and economic valuation assumptions will also be discussed. This method will be applied on a case study, the comparative LCA of bio-based polymers. Results show that the integrated impact allows discriminating these polymers, particularly with both the choice of the crop type and the production region.

**634 Historical Life Cycle Assessment of the Carbon Intensity of Oil Sands Production: 1970-2010** J.G. Englander, A.R. Brandt, Stanford University / Energy Resources Engineering. As one of the largest global oil resources, there has been considerable interest in the Alberta Oil Sands as an energy resource. However, as policies regulating the CO<sub>2</sub> intensity of fuels, such as California's Low Carbon Fuel Standard (LCFS) as well as protests surrounding the approval of the Keystone XL pipeline appear to indicate, a challenge to oil sands development may exist due to the comparatively more energy intensive and environmentally damaging extraction techniques required for extracting oil from the oil sands. Using data from Alberta's Energy Resources Conservation Board (ERCB), this paper builds a time-varying life cycle assessment model showing historical trends of greenhouse gas (GHG) intensity from oil sands oil production, from surface mining, in-situ production, upgrading and refining. Primary energy consumption data are collected for all energy sources including natural gas, coke, coal, and electricity, as well

as data on synthetic crude oil (SCO) and raw bitumen production for mining and upgrading operations, as well as steam to oil ratios (SOR), which are used to estimate in-situ production energy consumption. Interpolation techniques are developed to augment some missing data. Using these data, life cycle embodied energy and GHG emissions are estimated per unit of refined fuel produced ( $\text{gCO}_2/\text{MJ}$  Gasoline) from 1970 to 2010. Results suggest that as new technologies have been deployed, energy consumption per unit of liquid fuel produced has declined and there has been a shift to less carbon-intensive primary fuels. Between the beginning of commercial operations until the start of project expansion, the two largest oil sands projects, Suncor and Syncrude, experienced decreases in energy intensity of 56 and 61% respectively (measured as primary energy consumed per unit of SCO), which corresponded to decreases in carbon intensity of 50 and 55% ( $\text{gCO}_2/\text{MJ}$  SCO). However, these gains in efficiency have been attenuated by recent rapid expansions of some mining projects as well as a maturing of technology, which has largely halted the rate of emissions improvement after the year 2000. There are also considerable uncertainties regarding the contribution of emissions from tailings ponds, land use change, and other fugitive emissions.

**635 Assessing the impacts of abiotic resource use: application to electric vehicles batteries** V. De Bruille, CIRAIG – École Polytechnique de Montréal / Chemical Engineering; C. Bulle, CIRAIG – École Polytechnique; O. Jolliet, University of Michigan / School of Public Health, Dpt of Environmental Health Sciences; T. Dandres, CIRAIG – École Polytechnique; C. Gaudreault, NCASI Canada; R. Samson, CIRAIG – École Polytechnique. In this study, a new assessment method is proposed to assess the impacts from resources use, as none of the previously existing methods is considered mature enough to be recommended in the ILCD handbook. Based on resource functionality, future abundance and the financial consequences of the use of resources, this new approach allows impact monetisation. This allows considering an effect factor expressing to what extent the extraction of a certain amount of abiotic resource forces one of the future users to pay a different amount to keep the same functionality of the resource as it becomes scarcer. The effect factor is multiplied by a competition factor, which is a material competition scarcity index (MACSI) varying between 0% and 100%, defined to assess the competition. The MACSI is based on the “remaining years of availability”: the ratio between resource consumption and available known stock (including anthropogenic stock), representing the years left before total dissipation of a resource at current dissipation rate. Resource dissipation is the amount used from which recycling and reuse is subtracted. This choice was made considering that resource will remain (partly-) functionally equivalent, even after the end of a product life through reuse or recycling. Competition factors of about 100 minerals, for which remaining years of availability vary from 25 years (e.g. silver) to over 4000 years (e.g. perlite), are proposed as mid-point indicators for resource use, considering different time perspectives. The current project aims also at identifying the main affected users and include their adaptation capacities into the competition factor as well as the cost raise for competing users to adapt themselves to functionality loss. In order to illustrate the approach, it has been applied to metallic resources used in electrical vehicles batteries to compute impacts on natural resources depletion. Results have been compared with the impacts obtained with other LCIA methodologies to put in perspective advantages and drawbacks of each of them and see to what extend results obtained are consistent when applying different approaches showing different degrees of sophistication. Inconsistencies between methodologies are put into light as the ranking between contributors to the impact differ from one methodology to the other.

**636 How the New National Life Cycle Impact Assessment Standard Handles Resource Depletion** A. Foster, K. Killpack, T. Schultz, Scientific Certification Systems. The new national Life Cycle Assessment (LCA) standard currently undergoing finalization provides protocols for calculating results for category indicators related to the depletion of resources. This presentation will cover the specific methods described in this standard for quantifying resource depletion under four basic categories: energy resources (biotic and abiotic), water resources, minerals and metals, and non-energy related biotic resources. Calculating resource *depletion* takes into consideration more than the level of *consumption*. For each impact category in this group, the ratio of overall use of the resource to the resource reserve base being depleted is established as a measure of the resource's relative abundance, using resource depletion characterization factors (RDFs), and the rate of accretion and recycling is also taken into account. Aside from energy

resource depletion, which can be aggregated on an equivalent unit of oil basis, the aggregation of material abiotic or biotic resources into a single category indicator result is not allowed in this standard because it violates the basic ISO 14044 tenet prohibiting combining different environmental mechanisms under one indicator. For example, aggregation of copper and steel resource depletion into a single category indicator would mask the fact that copper is becoming scarce globally, while the world still has an abundance of steel reserves both in standing inventory and ore reserves, especially when the high recycling rate of steel is factored in. Examples from several case studies in the green building sector and energy sectors will be presented to demonstrate how resource depletion calculations are conducted.

**637 Building reliability, credibility and consensus in Life Cycle Impact Assessment** O. Jolliet, The University of Michigan / School of Public Health, University of Michigan / Environmental Health Sciences, School of Public Health; B.W. Vigon, SETAC; J. Bare, US-EPA; R. Frischknecht, ESU-Services Ltd; T. McKone, University of California, Berkeley, University of California and Lawrence Berkeley National Laboratory, University of California, University of California and Lawrence Berkeley National Laboratory, University of California. In the last five years important progresses have been made in Life Cycle Impact Assessment (LCIA): international collaboration has led to the USEtox consensus model for the human toxicity and ecotoxicity categories, with compatible intake fractions for respiratory inorganics, characterization factors for indoor air emissions and ionizing chemicals as well as metals. The resource use categories have been framed for water use, land use, minerals and energy, looking at consumptive use and competition between various users and resource quality. Recently, the scope has broadened to aim at a world coverage within several efforts such as the LC-IMPACT European project, the IMPACTworld+ method, the Sustainability consortium effort, the next version of the Japanese LIME method, and further development within US-EPA. Characterization factors are becoming increasingly available for various continents and/or world regions. In this context, these converging efforts and in more general the LCIA field would highly benefit to put resources from all interested teams worldwide in synergy towards evaluating the credibility, the reliability of LCIA methods and pursue coordinated research efforts to further develop LCIA scientific methods as a basis for international consensus. This presentation will present a new worldwide effort in LCIA carried out in interaction with the UNEP-SETAC Life cycle Initiative and involving stakeholders worldwide. Building on previous LCIA works, we will identify in a first step the Life Cycle Impact indicators to prioritize based on environmental problems that matter most, problem raised by stakeholders and the quality of existing LCIA methods. An initial scoping workshop will take place in conjunction with the SETAC-Europe meeting in Glasgow to set these priorities, analysing the uncertainty, reliability and credibility of best presently available methods and identifying critical gaps, research and developments needed towards building consensus. Effort towards consensus will then be carried out for a first set of five indicators, eventually leading to their presentation within an ulterior Gordon conference, also involving in the process expert scientist from specific fields related to these indicators. This process represents an important step forward towards enhancing the quality and consensus on a default LCIA method and indicators.

**638 Reclaiming Biomass for use as Fertilizers from Algal Biofuel Production** C.L. Antaya, W. Barr, A.E. Landis, Arizona State University / School of Sustainable Engineering and The Built Environment. Algae feedstocks represent the next generation for sustainable biofuels due in part to their ease of cultivation; algae utilize sunlight and nutrients nitrogen phosphorus, primarily, as well as carbon dioxide, doubling cell mass in a couple of hours. These systems present opportunities for industrial synergies utilizing waste carbon dioxide as well as nutrient rich municipal or industrial wastewater for production and have minimal impact agricultural lands unlike specifically land-derived biofuels. Despite this, the process of producing algal biofuels generates substantial waste biomass post-lipid extraction. Sustainability assessments now focus on reclaiming the downstream biomass as a co-product, rather than discarding it as waste. A small number of applications for the use of waste algae biomass have been proposed, including animal feed and biofertilizers. Prior research has suggested that nutrients, nitrogen and phosphorous, contained in the biomass are readily available for use as a soil amendment without further modification. While preliminary studies conclude that algal biofertilizers are slow-releasing nitrogen fertilizers, they may also contribute significantly to eutrophication impacts.

This presentation evaluates the bio-available nutrient content of waste algae biomass and utilizes a life cycle assessment framework to estimate the environmental impacts related to reclaiming waste algal biomass as a biofertilizer. The system boundaries for this study include the algae cultivation system via a photobioreactor, while agricultural fertilizer offsets were calculated for the use of the reclaimed biomass as a biofertilizer product. Municipal wastewater and agricultural fertilizers were compared as different sources of nitrogen and phosphorous resources for algal growth. The results presented in this study include the environmental impacts from reclaiming the waste biomass as a biofertilizer, the nutrient content of the resulting biofertilizer and an assessment of agricultural offsets as an outcome of algal biofertilizer usage.

### **639 Evaluation of Polyethylene Passive Samplers to Estimate Deep Water PCB Concentrations at the Palos Verdes Shelf Superfund Site**

R.M. Burgess, U.S. EPA / Atlantic Ecology Division, National Health and Environmental Effects Research Laboratory, US EPA, U.S. EPA, U.S. Environmental Protection Agency / Ord/Nheerl-Atlantic Ecology Div, U.S. EPA / Ord/Nheerl-Atlantic Ecology Div; R. Lohmann, University of Rhode Island / Graduate School of Oceanography, University of Rhode Island; P. Luey, Narragansett Bay Program; M. Charpentier, Raytheon; C. White, U.S. EPA. The Palos Verdes Superfund site is located in over 50 meters of water on the continental shelf and slope off the coast of southern California (USA). The site includes 27 km<sup>2</sup> of seabed contaminated over several decades by municipal treatment plant effluent discharged via outfall pipes at a depth of 60 meters. Contaminants of concern include industrial chemicals like polychlorinated biphenyls (PCBs) and the pesticide DDT and its degradation products. Planned remediation of the site includes capping of erosive areas designated as hot spots with clean capping material. A risk to the Palos Verdes system is contaminated sediment resuspension as capping material is placed on the seafloor. Resuspension of sediments and resulting desorption of dissolved contaminants into the water column could cause aqueous concentrations of PCBs and DDTs to exceed marine water quality criteria (WQC). For this preliminary pre-remediation investigation, passive sampling polyethylene devices (PEDs) were deployed on current meters one to five meters above the seabed with various levels of sediment contamination for three to six months at seven stations. The objectives of these measurements were to (1) evaluate the use of passive samplers in deep waters and (2) quantify baseline concentrations of dissolved PCBs in water above the sediments and assess their transport from the site. PEDs absorb contaminants from the water column and provide a robust method for estimating dissolved contaminant concentrations at very low levels conventional water sampling procedures would not be able to detect. At this site, PEDs functioned well as monitoring tools with concentrations of total PCBs ranging from 60 to 400 ng/g polyethylene which corresponds to estimated water column dissolved concentrations of 90 to 1000 pg/L. Mean flow measured by the current meters is along the shelf toward the northwest. Water column PCB concentrations were highest above and to the northwest of the most contaminated sediments, suggesting PCBs are partitioning into the water column from the more contaminated sediments and being transported northwest from the Superfund site by the mean current. These preliminary baseline data, along with more extensive water column baseline monitoring data, will be compared to similar measures taken during and after the remediation to access the magnitude of contaminant release and probability of exceeding WQC.

### **640 Using two types of passive samplers to measure dissolved persistent organic pollutants in the water column of the Palos Verdes Shelf Superfund site**

L.A. Fernandez, U.S. Environmental Protection Agency / Office of Research and Development; W. Lao, Southern California Coastal Water Research Project (SCCWRP); K.A. Maruya, Southern California Coastal Water Research Project, SCCWRP; C. White, U.S. Environmental Protection Agency / Region 9; R.M. Burgess, U.S. EPA / Atlantic Ecology Division, National Health and Environmental Effects Research Laboratory, US EPA, U.S. EPA, U.S. Environmental Protection Agency / Ord/Nheerl-Atlantic Ecology Div, U.S. EPA / Ord/Nheerl-Atlantic Ecology Div. Passive sampling methods were used to deduce water concentrations of persistent organic pollutants (POPs) in the vicinity of a marine Superfund site on the Palos Verdes Shelf, California, USA. This type of sampling can detect POPs at concentrations that are undetectable by conventional sampling techniques. Pre-calibrated solid phase microextraction (SPME) fibers and polyethylene (PE) strips that were pre-loaded with performance reference compounds (PRCs) were deployed for 32 d along an 11 station

gradient at bottom, surface and mid-water depths. Retrieved samplers were analyzed for DDT congeners and their breakdown products (DDE, DDD, DDMU, and DDNU) and 43 PCB congeners using GC-EI- and NCI-MS. SPME samplers were assumed to have reached equilibrium during the deployment. PRCs were used to calculate compound specific fractional equilibration achieved *in situ* for the PE samplers, using both molar volume adjustments (MVA) and a mass-transfer model with best-fit boundary layer estimation. The highest observed concentrations were for p,p'-DDE, with levels of 2200 pg/L and 990 pg/L deduced from PE and SPME, respectively. The difference in these estimates could be largely attributed to uncertainty in equilibrium partition coefficients or unaccounted for disequilibrium between samplers and water. The concordance between PE and SPME estimated concentrations for DDE was high ( $R^2 = 0.95$ ). PCBs were only detected in PE samplers, due to the much larger polymer mass deployed. Near-bottom waters adjacent to and down current from sediments with the highest bulk concentrations exhibited aqueous concentrations of DDTs and PCBs that exceeded Ambient Water Quality Criteria (AWQC) for human and aquatic health. Continued passive sampling is intended for this site to determine the effectiveness of planned remedial activities taken against the most highly contaminated surface sediments.

### **641 Measuring sediment porewater organochlorine concentration gradients on the Palos Verdes Shelf using passive samplers**

L.A. Fernandez, U.S. Environmental Protection Agency / Office of Research and Development; W. Lao, Southern California Coastal Water Research Project (SCCWRP); K.A. Maruya, Southern California Coastal Water Research Project, SCCWRP; C. White, U.S. Environmental Protection Agency (retired); R.M. Burgess, U.S. EPA / Atlantic Ecology Division, National Health and Environmental Effects Research Laboratory, US EPA, U.S. EPA, U.S. Environmental Protection Agency / Ord/Nheerl-Atlantic Ecology Div, U.S. EPA / Ord/Nheerl-Atlantic Ecology Div. The Palos Verdes Shelf Superfund site is in over 50 meters of water on the continental shelf and slope off the coast of southern California (USA). The site includes over 25 km<sup>2</sup> of sediments contaminated over several decades by municipal treatment plant effluent discharged via outfall pipes at a depth of 60 meters. Contaminants of concern include polychlorinated biphenyls (PCBs) and the pesticide DDT and its degradation products (e.g., DDE). Planned remediation of the site includes capping of erosive areas designated as hot spots with clean material. Selection of the specific sections of the site to cap will be based, in part, on identifying areas that represent the greatest risk of contaminant release into the bottom waters. Mechanisms of dissolved contaminant release include diffusion from the sediment interstitial water into the bottom waters. Because of the site's excessive depth, determining which areas to cap is challenging. Passive sampling represents a possible technology for measuring dissolved contaminant gradients between the interstitial waters and bottom waters as well as assessing the effectiveness of capping. In this study, three types of samplers (polyethylene and polyoxymethylene strips, and solid phase micro extraction fibers) were deployed in the sediments and bottom waters of Palos Verdes Shelf Superfund site. The deployment platform was designed to allow sampling of the sediment interstitial waters at 2 cm increments to a depth of up to 20 cm, and to sample the bottom water in the 20 cm above the sediment-water interface. The performances of the three types of samplers were compared. The four platforms deployed performed very well and were successfully recovered after approximately 30 days. Among the sites sampled was an area where a pilot, sand cap was placed several years ago to test the efficacy of the remedial plan. At this station, interstitial water concentrations of DDE increased with depth in the top 4 cm, remaining relatively constant for the next 10-12 cm, then began to increase again. DDE concentrations in the bottom water at this station are slightly lower than those in the mid-depth sediments. To assess the release of dissolved contaminants, and the effectiveness of capping, these results are compared to similar interstitial water concentration gradients at other stations on the shelf where no capping has occurred.

### **642 Deposition and Accumulation of Emerging Contaminants in the Sediments of the Palos Verde Shelf, California**

M.G. Cantwell, / Atlantic Ecology Division; D.R. Katz, US EPA / Atlantic Ecology Division, U.S. EPA / ORD\NHEERL\AED; J. Sullivan, US EPA; R.P. Eganhouse, USGS; M.M. Perron, NRC/EPA; R.M. Burgess, U.S. EPA / Atlantic Ecology Division, National Health and Environmental Effects Research Laboratory, US EPA, U.S. EPA, U.S. Environmental Protection Agency / Ord/Nheerl-Atlantic Ecology Div, U.S. EPA / Ord/Nheerl-Atlantic Ecology Div. The



Palos Verdes shelf is located off the Southern California coast and has been receiving wastewater effluents from the Los Angeles County municipal sanitation Districts since 1937. Currently, a large segment of the Palos Verdes shelf is listed as a U.S. EPA Superfund site due to long-term discharge of DDT and PCBs which have resulted in highly contaminated sediments. Although the fate and behavior of these "legacy contaminants" has been extensively studied, little information exists on the presence and historical trends of newer classes of pollutants that are classified as contaminants of emerging concern. Antimicrobial compounds and brominated flame retardants, both of which are used extensively in consumer based products, are two such classes of chemicals. In this study, triclosan, a phenolic antimicrobial additive, and polybrominated diphenylethers (PBDEs), which are used as flame retardants, were measured in a 50-cm long sediment core collected from the Palos Verdes shelf in 2009. Measurable triclosan is present to the base of the core and increases progressively up core to a maximum of 29 ng g<sup>-1</sup> at 5 cm depth, which is consistent with contemporary levels observed in sediments at other marine locations impacted by wastewater effluents. Measurable PBDEs first appear at a depth of 43-45 cm and are comprised of major congeners associated with penta formulations (e.g., PBDE-47, 99, 100), and decabromodiphenylether (PBDE-209). The congeners used in the penta-PBDE formulation account for the majority of PBDEs present throughout the length of the core. In contrast, PBDE-183, a major component of the octa-PBDE technical mixtures is present only at extremely low concentrations. At 31 cm depth, PBDE-209 is the most abundant congener measured, with concentrations increasing up core, demonstrating its increasing use over time. Within 5 cm of the sediment-water interface, all congeners show a decline suggesting that regulations in California banning the use and production of PBDEs along with voluntary phase outs may be responsible for reducing the amount of PBDEs being released to surficial sediment of the Palos Verdes Shelf.

**643 Assessing Bioavailability of DDTs and PCBs in Palos Verdes (PV) Shelf Superfund Sediments** J. Voelker, University of California, Riverside / Department of Environmental Sciences; L. Bao, University of California Riverside / Chinese Academy of Sciences, University of California, Riverside / Department of Environmental Sciences; R. Lavado, University of California Riverside / Department of Environmental Sciences, University of California Riverside / Department of Environmental Science; J. Gully, Environmental Scientist / Ocean Monitoring & Research Section, Supervising Environmental Scientist / Ocean Monitoring & Research Group; D. Schlenk, University of California-Riverside / Department of Environmental Sciences; J. Gan, University of California Riverside. Sediments from Palos Verdes (PV) Shelf are heavily contaminated with DDT and metabolites and to a lesser degree, various PCB congeners. Given that the contamination occurred decades ago and that the contamination spanned over many years in the last century, it is highly likely that the contaminant bioavailability has diminished and that the bioavailability may also vary spatially. Although extensive monitoring has been done in the characterization of PV Shelf sediments, the relative bioavailability of DDTs and PCBs is unknown. Using sediment samples taken from the 8C and 6C sites, we have evaluated the bioavailability of DDTs and PCBs using several approaches, including Tenax extraction, carbon normalization, solid phase microextraction and stable isotope exchange. In addition, we have modified the 8C sediment through amendment of activated carbon or sand to simulate remediation treatments. The bioaccumulation of selected DDT derivatives and PCB congeners was simultaneously measured using the marine polychaete worm *Neanthes arenaceodentata*. Results indicate that DDTs and PCBs in the marine sediment display much reduced bioavailability as compared to freshly spiked compounds, and that amendment of carbon or sand greatly altered the bioavailability.

**644 A Multiple Measures Approach for Screening for Contaminant Effects in Coastal California Fish** K. Kelley, California State University, Long Beach / Environmental Endocrinology Laboratory, California State Univ, Long Beach; J.A. Reyes, Pacific Coast Environmental Conservancy. In our studies in coastal California, including in the central Southern California Bight (SCB) and offshore of Palos Verdes (PV), it is clear that different kinds of physiological effects observed in fish reflect specific types of environmental contaminants. Since different types or classes of chemicals typically act through distinct pathways in organisms, the development of multiple-measures approaches (simultaneously applied in a single sample) have strong potential to serve as screening and diagnostic tools to predict

types or classes of environmental constituents and their specific physiological and health effects. It has therefore been the goal of our studies to develop multiple-measures approaches using proteomics technologies combined with measures of endocrine and physiological status. In coastal fish, proteomes of liver and other tissues are being characterized in relation to residence near regional wastewater treatment facilities, contaminated superfund sites, and other locations, to discover protein biomarkers whose expression are altered in relation to contaminant exposures and endocrine system status (endocrine disruption). Since all parameters are measured within the same individuals, it is possible to evaluate contaminant exposures, effects, and endocrine system status using correlative and multivariate statistical approaches. Identification of protein and endocrine biomarkers and their expression differences in fish have pointed to specific changes in detoxification processes, oxidative stress response, hepatic fuel metabolism, altered signaling systems (endocrine, intracellular), and physiological changes relating to growth and reproduction. This multiple-measures approach is providing novel information on phenotypic effects in animals exposed to different kinds of environmental contaminants, from organochlorine pesticides, PCBs, PAHs, and others, currently present offshore of PV and the SCB. [Supported by NOAA/USC Sea Grant Program]

**645 Long-term, fine-scale movements of teleost fishes in the Palos Verdes Shelf Superfund Site and application for monitoring potential contaminant uptake** B.W. Wolfe, G.N. Teesdale, C.G. Lowe, California State University Long Beach / Department of Biological Sciences. Although the presence of organochlorine contaminants in commercially and recreationally targeted fishes is well documented, the relationship between habitat usage and contaminant uptake in fishes is poorly understood. New advances in acoustic telemetry technology have allowed for high resolution simultaneous tracking of multiple animals over extended periods of time in areas of high organochlorine contamination. We used Vemco VPS technology and traditional passive tracking to record fine-scale movements of 97 white croaker (*Genyonemus lineatus*) and 54 barred sand bass (*Paralabrax nebulifer*) across a 20 km<sup>2</sup> area encompassing the Palos Verdes Shelf Superfund Site (PVSSS) as well as other areas throughout the Southern California Bight (SCB), CA from July 2010 – September 2012. Site fidelity, home ranging behavior and habitat association of the three species were quantified with regards to benthic sediment organochlorine concentration. Barred sand bass showed higher site fidelity to non-spawning season home ranges on the shelf, whereas white croaker ranged over much greater areas. Over 40% of the white croaker tagged on the PVSSS were observed to move into Los Angeles Harbor, while 20% showed periods of extensive activity within the PVSSS. Nearly all tagged barred sand bass left their non-spawning season home ranges within the PVSSS to join known spawning aggregations throughout the SCB. These movement patterns suggest means by which highly contaminated fish have been sampled in areas outside the PVSSS and likely explain the high variability seen in organochlorine body burdens of fish collected at these locations. These data provide a crucially needed understanding of the movements of these economically and ecologically important fishes, especially with regards to contaminant uptake, within the PVSSS and throughout the SCB.

**646 A Bioaccumulation Model of the Effects of DDTs and PCBs at the Palos Verdes Shelf: A Tool to Determine Risk and in Support of Remediation** E. Byron, CH2M Hill / Environmental Services, CH2M Hill; C. Arenal, CH2M Hill. CH2M HILL developed a GIS-based food chain exposure model for DDT and PCB risk to fish, marine mammals, and birds of the Southern California Bight in order to predict ecological risk near the Palos Verdes Shelf (PVS). The model was used as a key component of the Ecological Risk Assessment for current conditions for the PVS area in the RI/FS. Risks from sediment exposure were evaluated for invertebrates, fish, sea lions, and four species of birds. The model was used as a primary tool in demonstrating risk and in evaluating human health and ecological benefits of remedial action scenarios. The key underpinning of the model was the extensive, Southern California bight-wide and Palos Verdes-specific sediment and fish tissue concentration estimates for organochlorine contaminants. This extensive database not only provided direct evidence of risk for the Ecological Risk Assessment, but it allowed the creation of a significant, spatially-based relationship between fish tissue and bulk sediment concentrations for both DDTs and PCBs. Neither regression relationship was significantly improved by organic carbon or lipid normalization of the data. The quantitative relationship between sediment and fish

tissue concentrations allowed the use of the extensive bight-wide sediment database to provide an exposure estimate of bight-wide fish. Once fish were estimated, mechanistic tissue relationships based on diet, ingestion, and assimilation by species could be used to evaluate potential uptake and risk to seabirds, bald eagles, and marine mammals. The known, local foraging ranges of the key receptor species (bald eagle, sea lion, brown pelican) were used to link exposure and risk.

**647 Assessment of PBTs in the EU: A critical review and proposed evaluation scheme with reference to plant protection products** K. Solomon, University of Guelph / School of Environmental Sciences, University of Guelph / Centre for Toxicology, School of Environmental Sciences; M. Matthies, University of Osnabrueck / Institute for Environmental Systems Research; M. Vighi, University of Milano Bicocca / Department of Environmental Sciences, University of Milano / Department of Environmental Sciences. A number of international and national programs classify substances that are persistent (P or very P), bioaccumulative (B or very B), toxic (T), or have the potential for long-range transport (LRT). The oldest of these programs is the Stockholm Convention on Persistent Organic Pollutants (POPs). More recent programs address PBTs for chemicals in general (REACH EC253/2011) and plant protection products (PPPs EC1107/2009). However, these programs used different criteria for classification. We critically reviewed the criteria and process used in the classification of pesticides and noted that EC 1107/2009, in contrast to the Stockholm Convention or REACH, offers no process for carrying out a further more refined assessment of those pesticides that are identified as having PBT properties. Thus, in contrast to REACH, screening criteria are used for final-step management decisions. Guidance on the selection of data is not provided and the criteria used are unclear. For example, no guidance is given as to how the half-lives in soil, water, and sediment should be derived and the term "half-life" is not clearly defined (e.g.,  $DT_{50}^{dissipation}$  or  $DT_{50}^{degradation}$ ). Large amounts of useful data on environmental and toxicological properties are available for PPPs but most of this is not used in the classification. For example, photolysis in water, water-sediment, and on soil, an important environmental degradation process that is particularly relevant to pesticides, is not used as a screening criterion. The criteria for bioaccumulation and toxicity appear to be focused only on aquatic ecosystems and do not address movement from the aquatic to terrestrial species or effects in plants. The classification process under EC 1107/2009 could be made more efficient and reduce false negatives and positives if a formal weight of evidence approach was applied to multiple lines of evidence. This paper presents these ideas and how they can be incorporated into the framework for classification to better classify pesticides in terms of persistence, bioaccumulation, and toxicity.

**648 Ecotoxicological evaluation of soils and sediments of reference and satellite sites of the Mexican National Monitoring Program (PRONAME)** M.D. Guzman Martinez, L. Elizalde Ramirez, A.L. Espejel Pina, I. Arellano Lopez, E. Sierra Hernandez, U.A.M. Iztapalapa / Hidrobiologia; A.D. Nava Montes, Instituto Nacional de Ecología / CENICA; P. Ramirez Romero, Universidad Autonoma Metropolitana / Hidrobiologia, U.A.M. Iztapalapa / Depto. De Hidrobiologia. Mexico is signatory of a variety of international environmental agreements and to comply with some of the commitments acquired a monitoring program was established in 2007. The National Monitoring Program (PRONAME) will monitor in a long term (> 25 years) persistent and bioaccumulative toxic substances in sites with different levels and types of anthropogenic influence. The objective of the present work is to present the results of some bioassays that complement the chemical analysis of two reference sites: Celestún and Manantlan Biosphere Reserves and three satellite sites: Yaqui and Bravo Valleys and Coatzacoalcos. Samples of soils and sediments were obtained and transported to the laboratory. Extracts were obtained through sonication and evaporation; these extracts were diluted in reconstituted fresh water to analyze their toxicity with the lettuce seed bioassay in which germination, root and hypocotyl length were evaluated. Also a direct contact toxicity test using Microtox® was applied to the 2010 samples. In the lettuce seed bioassays germination percentages were high and no toxicity was detected. Microtox® detected toxicity in a small group of samples from the reference sites (Celestun > Manantlan) and even though Coatzacoalcos is an industrial area and Valle del Yaqui an agricultural one, no sample showed toxicity. In contrast a good number of samples from Valle de Bravo were toxic, which is associated with the presence of excess nutrients from the untreated waste waters that this

system receives. To better this evaluation other bioassays are being analyzed as well as to start measuring biomarkers on different organisms at each of the sites.

**649 Enhanced Persistency Assessments: Putting the "bio" back into biodegradation testing** T.J. Martin, Newcastle University / School of Civil Engineering and Geosciences; A.K. Goodhead, Newcastle University / Civil Engineering and Geosciences; M. Daniel, A. Bartram, J.R. Snape, Brixham Environmental Laboratory; R.J. Davenport, Newcastle University / Civil Engineering and Geosciences. Regulatory emphasis has shifted recently towards hazard identification and prioritising chemicals which are persistent, bioaccumulative and toxic (PBT): since chemicals with these properties have previously been shown to be most harmful to human health and the environment. Biodegradation is one of the most important fate processes determining persistence and has historically been assessed using highly prescribed, stringent, standardised tests termed Ready Biodegradability Tests (RBTs). Whilst RBTs have typically formed the core protocol for developing regulatory guidelines for persistency and environmental exposure assessments, the stringent nature and high false negative incidence associated with the tests renders them unsuitable for prioritising on the basis of environmental persistence. Registration, Evaluation, Authorisation and restriction of CHemicals (REACH) guidance has recognised this and advocates the introduction of a new tier of modified and enhanced tests to enable a more effective prioritisation on persistence. Reliable extrapolation from small-scale systems to predict local and regional effects is dependent upon incorporating environmental realism into laboratory scale systems, with better replication of natural ecosystems, including the nature of the microbial populations present. Enhanced tests under REACH allow increases in inoculum density to environmentally equivalent concentrations and increases in test volume, both of which may contribute towards providing a more environmentally realistic microbial community composition and diversity. This presentation describes the effect of inoculum concentration and test volume on biodegradation outcome for a group of compounds with varying persistency in activated sludge (AS) and marine systems. Molecular techniques were applied to investigate the relationship between microbial diversity, its variation within test inocula and the impact on the probability of a positive biodegradation outcome. It is anticipated that this will allow for better predictions on biodegradation with the ultimate goal of using this knowledge to design more robust screening tests for persistence.

**650 Evolving Interpretation of Listing Criteria Leads to Confusion in Stockholm and LRTAP POPs Assessments** D.J. Kent, D. Graham, H. Estreicher, Keller and Heckman LLP. Two major international agreements used to identify and ban persistent organic pollutants have recently experienced an evolution in the interpretation of their listing criteria. The Stockholm Convention on POPs is a global treaty focused on "chemicals that remain intact in the environment for long periods, become widely distributed geographically and accumulate in the fatty tissue of humans and wildlife." Similarly, the goal of the Long Range Transboundary Air Pollution POPs protocol goal is to "eliminate any discharges, emissions and losses of POPs." Both protocols have specific criteria that chemicals must meet to be listed, including evaluation of persistence, bioaccumulation and biomagnification potential, and the ability to be transported long ranges to remote regions. Based on these criteria there are about two dozen chemicals that have been severely restricted or banned, with additional chemicals currently being considered for listing. Recently the governing criteria have been more liberally interpreted such that when the parent chemical does not meet the criteria it may still be considered for listing based on degradation and metabolic products. Such expansion of the criteria has caused some confusion, both with the member state parties tasked with making recommendations and with the manufacturers and downstream users of chemicals under investigation. This confusion can be especially onerous when the degradation product is used to list a parent chemical even when the same degradation product may actually be sourced from a completely different parent chemical that is already well known to long-range transport. This presentation looks at some of the key issues and proposes some harmonization standards that can be applied to ensure consistent science-based listing procedures. These procedures include the need to use reliable data, specific criteria, appropriate long-range transport models, and effective use of biomonitoring information. A key recommendation will be to have procedures in place to fully recognize and assess other sources of degradation and metabolism products

when those products are used to assert listing when the parent chemical does not meet the listing criteria.

**651 Has the time come to revisit, revise and expand PBT criteria?** D. Mackay, Trent University, Trent University / Environmental Studies; J.A. Arnot, ARC Arnot Research & Consulting Inc., University of Toronto Scarborough / Department of Physical and Environmental Sciences. The assessment of the relative hazard of chemicals using PBT and LRT criteria has been largely successful, however, there is little doubt that it can be improved to better identify high priority substances. The expressed need for international harmonization provides an opportunity to revisit and refine the criteria in the light of advances in environmental science. We discuss aspects of the four criteria. Persistence (P) is well expressed by media specific half lives and an overall multimedia persistence, but the use of 'bright line' values can be suspect given the high variability and uncertainty inherent in half lives. There is a need to determine standardized 'recovery' times. Many "non P" substances can also be of concern when exposure is in the 'near field' close to sources. Bioaccumulation (B) presents challenges because, while BCFs can be measured reproducibly, they are not always ecologically relevant. A new dietary uptake BAF/BMF test may provide improved methods of integrating BCFs, BAFs, BMFs and TMFs for fish, mammals and birds using models. Models show the critical need for better estimates of biotransformation rates. Toxicity (T) expressed as LC50s lacks credibility because hydrophobicity creates an illusion of toxicity. Delivered doses, critical body residues or chemical activities are clearly preferable for comparing and ranking toxicities. Chemical specific differences in Long Range Transport (LRT) are well described by the Characteristic Travel Distance (CTD), but the CTD can be misinterpreted and it may fail to include changes in geography and climate that inevitably occur over long distances. The effect of emission rates on exposures in remote regions should be addressed. Finally, there is a need to integrate these metrics into a single 'score'. Rather than simply 'adding' the individual pass/fail criteria, we suggest estimating relative exposures and risks in a standard evaluative environment to both humans and ecosystems using the information contained in the PBT-LRT criteria. Multimedia models of fate and exposure are becoming available and more credible that can address this task, including uncertainty analyses. It is hoped that these suggestions will contribute constructively to improved identification of chemicals of highest priority for further evaluation, reduction of uncertainties and possible regulation including considerations for uncertainty.

**652 Prospects for measuring persistence in the field using chemical benchmarking** H. Zou, M. McLachlan, Stockholm University / Department of Applied Environmental Science (ITM); M. Macleod, Stockholm University / Applied Environmental Science (ITM). Persistence is one of the hazard properties of a chemical that is evaluated in regulatory strategies at national and international levels. Persistence assessments are based on measurements in the laboratory, which could induce great uncertainties when extrapolating to natural conditions. There is no established method for assessing persistence from measurements of chemicals in the field. In this study, the potential of using a benchmarking technique to assess the persistence of chemicals in real lake systems was evaluated by conducting model experiments with a multimedia chemical fate model. The benchmarking technique involves measurements of a set of benchmark chemicals with known fate processes in the lake simultaneously with the test chemicals of interest. The set of benchmark chemicals includes persistent substances that are: 1) non-volatile and water soluble and thus removed by advection in water; 2) very hydrophobic, and thus removed by burial in sediments. These substances are tracers of removal processes that are properties of the lake system. For hydrophobic test chemicals that are removed mainly by burial compared with water advection and volatilization, additional benchmark chemicals with similar hydrophobicities as the test chemicals are required. We used a multimedia fugacity model to establish the ranges of chemical and lake-system properties where the benchmark technique could be applied to estimate persistence from field measurements. Chemical space plots defined by logarithms of octanol-water ( $\log K_{ow}$ ) and air-water ( $\log K_{aw}$ ) partition coefficients of test chemicals were explored, along with a range of lake characteristics such as depth, water residence time, suspended solid concentrations and burial rates. The model experiments show that the benchmarking technique is likely to allow persistence to be measured in the field for chemicals that are not highly associated with particles in lakes with a wide range of characteristics. For particle-associated chemicals that are mainly removed by burial compared with advection and volatilization,

persistence can be best determined in lakes with high burial rates. Uncertainty analysis of model scenarios indicates that the relationship of organic carbon-water partition coefficient  $K_{oc}$  and  $K_{ow}$  determines  $K_{ow}$  boundaries for chemicals whose persistence can be determined using the benchmarking technique. A 'recipe' of how the benchmark technique could be applied in practice will be presented.

**653 Relationship between the BCF, BAF, and the TMF** F.A. Gobas, Simon Fraser University / School of Resource & Environmental Management, Faculty of Environment; A.R. Brisebois, Simon Fraser University / Resource and Environmental Management; H.A. Leslie, Institute for Environmental Studies, VU University; P. Leonards, Institute for Environmental Studies, VU University / Chemistry & Biology. A reasonable degree of consensus exists among bioaccumulation scientists on the use of Trophic Magnification Factors (TMF) as "conclusive" evidence of the bioaccumulative nature of chemicals in the environment. However, regulatory criteria to determine whether a substance is bioaccumulative in most jurisdictions rely on Bioconcentration Factors (BCF), measured in laboratory tests. The BCF is not an ideal measure of bioaccumulation because they do not account for chemical biomagnification via trophic transfer or biodilution. Also, BCF tests are expensive and lengthy, and require the use of many animals. In this paper, we present the final results from laboratory, field, and modeling studies aimed at testing the hypothesis whether the BCF is a good predictor of the TMF. Our studies involved (i) the compilation of BCFs and calculation of BAFs for a range of potentially bioaccumulative substances through literature studies and calculations; (ii) a field study of the food-web bioaccumulation of the same substances in Western Scheldt Estuarine food-web aimed at measuring TMFs in food-webs containing both water and air-breathing organisms; (iii) a modeling study aimed at formulating and exploring the relationship between the BCF and the TMF. We conclude that the BCF can be a useful predictor of the TMF for a range of chemicals with specific chemical characteristics (i.e. fat soluble substances) in an estuarine ecosystem. We also conclude that there are two major types of errors where the BCF does not provide accurate information about the bioaccumulative nature of chemicals in the environment. The first error deals with substances that show a relatively low BCF but have a high TMF such as certain per-fluorinated and brominated compounds. The second error is for chemicals that exhibit a high BCF in laboratory tests, but which do not show biomagnification in food-webs and have a low TMF such as hexabromocyclodecane. Supported by model calculations, we propose a set of simple rules that may be useful in the interpretation of laboratory based bioconcentration factors in terms of their bioaccumulative nature in the environment. These rules may be helpful to regulators involved in bioaccumulation assessments and to scientists that apply bioconcentration studies to study the bioaccumulative nature of chemicals in the environment.

**654 Definition, calculation and measurement of POV and LRTP** D. van de Meent, A. Hollander, Radboud University Nijmegen / Institute of Wetland and Water Research. Overall persistence in the environment ( $P_{OV}$ ) and long-range transport potential (LRTP) have been proposed as operational measures of the "PBT-ness" of chemical substances, but have found little use in regulatory frameworks so far. We believe that this is because  $P_{OV}$  and LRTP do not clearly express entities that (i) can be readily understood or compared to agreed-on criteria, (ii) can be observed in the environment, and (iii) can be calculated from available physical and chemical substance properties. This study contributes to solving this (re)defining  $P_{OV}$  and LRTP in a way that allows validation by measurement and prediction from theory. We propose to define  $P_{OV}$  as the "average life time" of molecules in the environment and to define LRTP as the "average distance travelled" by molecules during their life in the environment. We show that  $P_{OV}$  is the ratio of the total time-integrated mass of the substance in the environment and the total mass released, and that LRTP is a function of the effective advection-, dispersion-, and degradation rates of the substance in the environment, which can be readily calculated from substance properties. We have tested these calculation rules by comparing model simulations of hexachlorobenzene (HCB), polychlorobiphenyls (PCB) and brominated diphenyl ethers (BDE) with reported field data for the same substances. We used the nested multimedia fate model SimpleBox in quasi-dynamic (level IV) mode to simulate time-varying concentrations in air, water and soil at various spatial scales. Our study shows that the average life time of HCB molecules, overall in the environment (air, water, soil) is 20 yr, that on average, PCB- and BDE molecules travel 1500 km into the Arctic during their residence in



the environment. These results are in fair agreement with published field observations of spatial and temporal trends for HCB, PCB's and BDE's. We feel that these results can easily be understood and that, defined, calculated and verified this way, politically acceptable criteria can be formulated. An understandable criteria of one generation (30 yr) seems a useful criteria for  $P_{OV}$ ; a limit value of LRTP could be set to the length scale of a typical remote area (1000 km).

**655 Antibiotic occurrence and antibiotic resistance in microbial communities of three water supply reservoirs** D. Barcelo, ICRA / Environmenta Chemistry, ICRA / Catalan Institute for Water Research, IDAEA-CSIC / Environmental Chemistry Department, Catalan Institute for Water Research (ICRA) / Environmental Chemistry Department; B. Huerta, E. Marti, M. Gros, S. Rodriguez-Mozaz, J. Balcazar, R. Marce, Catalan Institute for Water Research (ICRA). Pharmaceuticals are continuously released in the environment from discharges of wastewater treatment plants or through run-off from fields after application of sewage sludge and animal manure. They attract increasing attention as contaminants because of their potential threat to the ecosystems and public health, even at their very low concentrations and persistence rates. Among these compounds, antibiotics may pose a risk to the environment since they are designed to have a biochemical effect in the microorganisms, and thus can have a significant impact in the processes controlled by them in aquatic ecosystems. One of the greatest concerns about the presence of antibiotics in the environment is the escalation of antibiotic resistance as a consequence of chronic exposure of naturally occurring bacteria to sub-lethal doses of these compounds and the potential impact in public health. The objectives of this study were first to determine the levels of antibiotics in different environmental compartments (water, sediments and fish) of three reservoirs in Catalonia: La Llosa reservoir was chosen for its pristine condition, whereas Sau and Foix are respectively a moderate and a highly polluted reservoir. Antibiotic concentrations were correlated with the composition and functioning of the natural bacterial communities in the reservoirs as well as with the presence of antibiotic resistance genes, not only in water and sediment but also in fish intestine. A general chemical characterization showed a clear difference between the reservoirs in terms of eutrophication and chemical pollution, with exceptional high values in Foix reservoir. Foix samples showed the highest antibiotic content in water and sediment, as it was expected according to the pollution gradient of the target reservoirs. Load was particularly high for macrolides family, which is consistent with data related to antibiotic consumption last years. Antibiotic concentration found was consistent with the quantity of *qnrS* and *sulI* gene copies (index of antibiotic resistance for the quinolone and sulfamide family, respectively) found in the samples. Resistance genes were detected in Foix Reservoir at much higher levels than in the other two, less affected by anthropogenic contamination. The combination of chemical and biological data provided a broader perspective to assess the impact of antibiotics released into the environment and their potential effect as promoters of antibiotic resistance.

**656 Contaminants of emerging concern in source and treated drinking waters from 25 drinking water treatment plants: pharmaceuticals and waste indicators** E. T. Furlong, M.C. Noriega, U.S. Geological Survey / National Water Quality Laboratory; S.T. Glassmeyer, U.S. EPA / Office of Research and Development, U.S. EPA / NERL/MCEARD/CERB; D. Kolpin, USGS. The presence and distribution of contaminants of emerging concern (CECs) in water supplies, and potential consumer exposure to these CECs in drinking water, has been of substantial scientific and public interest. The U.S. Environmental Protection Agency and the U.S. Geological Survey jointly conducted a study of CECs in untreated and treated drinking waters across the United States, sampling ground- and surface-water sources prior to and after treatment processes commonly used to produce drinking water. The timing of sample collection was adjusted for the hydraulic residence time of the water-treatment plant to determine relative removal of CECs during treatment. An extensive quality assurance/quality control design was used to assess precision and accuracy of CEC determinations at environmental concentrations. More than 185 pharmaceuticals and anthropogenic waste indicators (AWIs) were determined by direct aqueous injection LC/MS/MS and by GC/MS, respectively. Pharmaceuticals were detected in both untreated and treated drinking water samples, generally ranging in concentration from 2.5 to 940 ng/L, with more frequent and higher concentrations occurring in untreated water samples. Metformin, tramadol, and carbamazepine were the most frequently detected

pharmaceuticals in untreated water samples at maximum concentrations of 730, 42, and 40 ng/L, respectively. Metformin, carbamazepine and cotinine were most frequently detected in treated water samples at maximum concentrations of 92, 17, and 16 ng/L, respectively. The AWIs caffeine, *N,N*-diethyl-meta-toluamide (DEET), and tri(2-butoxyethyl) phosphate were most frequently detected in untreated water at maximum concentrations of 130, 98, and 470 ng/L, respectively. Bromoform, caffeine, and DEET were the most commonly detected AICs in treated water samples at maximum concentrations of 3,300, 75, and 25 ng/L, respectively. Frequent high concentrations of bromoform likely are due to formation as a disinfection by-product (DBP). Preliminary conclusions from this study indicate that multiple detections of pharmaceuticals and AWIs at concentration below 1,000 ng/L occur in both untreated and treated drinking water. After treatment, detection frequencies and maximum concentrations typically decrease. Treatment processes evaluated in this study reduce many, but not all, determined compounds, although formation of degradates and DBPs warrants future investigation.

**657 Fate of  $^{14}\text{C}$ -Carbamazepine and  $^{14}\text{C}$ -Acetaminophen in Soils and Biosolids-amended Soils** J. Li, University of California, Riverside / Department of Environmental Sciences, University of California, Riverside; J. Gan, University of California, Riverside / Department of Environmental Sciences. The widespread occurrence of human and veterinary pharmaceuticals in the environment has raised growing public concerns because of the widespread use of drugs such as carbamazepine (CBZ) and acetaminophen (ACET) and their ubiquitous occurrence in the environment impacted by wastewater and biosolids. To date few studies have considered the fate and transformation of pharmaceuticals in the environment. The objective of the present study was to characterize the fate of radiolabeled  $^{14}\text{C}$ -carbamazepine and  $^{14}\text{C}$ -acetaminophen in agricultural soils. In soils,  $^{14}\text{C}$ -ACET was found to rapidly form non-extractable or bound residues. Within an incubation period of about 1 h, depending on the soil types, 10-57% of the applied radioactivity was non-extractable using a mixture of acetonitrile and water (70/30, v/v). In a silty clay soil, after 2 days of incubation, the combined total extractability was below 9.8%. The extractability of  $^{14}\text{C}$ -ACET continued to decrease throughout the incubation period. At the end of the incubation period, less than 5% of the applied radioactivity was extractable using the optimized extraction procedures. It is likely that most of the bound residues consisted of transformation products strongly bound or incorporated into the organic matter matrix. Depending on the soil, between 8.9% and 17.1% of  $^{14}\text{C}$ -acetaminophen was recovered as  $^{14}\text{CO}_2$  at the end of the incubation period, however, only 0.37 %-1.18 % of  $^{14}\text{C}$ -carbamazepine was mineralized. Enhanced mineralization was seen in the sandy clay loam or silty clay soil than in a medium loam soil. Soil sterilization and amendment of biosolids significantly decreased the mineralization rate of acetaminophen. This study clearly showed that ACET is readily deactivated through mineralization and formation of bound residues in soil and CBZ is more persistent in soil.

**658 Fate of pharmaceuticals in surface waters downstream of a municipal wastewater treatment plant** S. Bartelt-Hunt, University of Nebraska / Civil Engineering, University of Nebraska-Lincoln / Civil Engineering; D. Brown, G. Hunt, University of Nebraska-Lincoln; D. Snow, University of Nebraska-Lincoln / Water Sciences Laboratory, School of Natural Resources, University of Nebraska. Wastewater treatment plants have been proved to be ineffective in removing pharmaceuticals and as a result, these compounds are frequently detected in municipal wastewater effluents. In this study, the fate of 25 pharmaceuticals including veterinary and human antibiotics and non-prescription drugs was monitored in surface water downstream of a wastewater treatment plant outfall using Polar Organic Chemical Integrative Samplers (POCIS). To quantitatively evaluate pharmaceutical fate in the environment, uptake rates were quantified in the laboratory. Twenty new uptake rates were determined for compounds that had no previously reported literature values. In surface water, select pharmaceuticals were observed to persist for at least 1300 m downstream. Carbamazepine and DEET were persistent with average aqueous in-stream concentrations of 110 and 60 ng/L, respectively. First-order loss rates were determined for 25 pharmaceuticals in receiving waters and pharmaceutical loading rates to the environment were determined.

**659 Occurrence of emerging endocrine active chemicals in wastewater effluents: synthetic steroids and thyroid disruptors** R. Marfil-Vega,

ORISE Postdoc at US EPA / ORISE Research Participant to the National Risk Management, University of Cincinnati, ORISE Postdoctoral Fellow, ORD, US EPA, ORISE Postdoc at US EPA; W. Brashear, PTS; K. Tadele, SSC at US EPA; M.A. Mills, U.S. EPA, U.S. EPA / National Risk Management Research Laboratory, ORD, US EPA / Remediation and Pollution Control Division, NRMRL, U.S. EPA / Office of Research & Development. Endocrine disruption encompasses the disturbance of various functions of living organisms, including metabolism, growth and development, tissue function, and mood. Affected metabolic pathways include, but are not limited to, steroidogenesis and nuclear receptors for steroid hormones (estrogens, androgens, and progestins) and thyroid homeostasis. Several classes of Endocrine Disrupting Chemicals (EDCs) with estrogenic effects have been extensively monitored during the past decade in wastewater and surface waters by the targeted chemical analysis. More recently, the attention of the scientific community has expanded to (anti)-androgenic compounds. Much less effort has been focused on the presence of progestins, agonists of the progesterone receptor, and thyroid hormones in the environmental waters. A reason for this could be that progesterone (natural progestin) is rapidly degraded during wastewater treatment, but the synthetic progestins are hypothesized to be more recalcitrant. Furthermore, the synthetic progestins are used for oral contraception at a higher amount than estrogens. Therefore, there is an elevated potential risk for progestins to reach the environment from wastewater treatment plant discharges. In regard to the thyroid hormone metabolism, its complexity allows for structurally diverse chemicals to disrupt different biological endpoints. Up to date, very limited information is available on the presence of thyroid disruptors, both from natural and industrial origin, in environmental waters. In the current work, we will be presenting the validation of an analytical method to monitor progestins and selected thyroid disruptors, as part of an on-going multilaboratory and interdisciplinary effort to evaluate the endocrine disrupting potency of wastewater effluents. Selected compounds were analyzed by Solid Phase Extraction followed by UPLC/MS/MS in effluents collected at four wastewater treatment plants during three different sampling campaigns. The data on the occurrence and concentrations will be reported.

**660 The relative contribution of humans, livestock and wildlife to the mass of 17 $\beta$ -estradiol and testosterone released to the environment** C. Hassinger, ARCADIS; P. Anderson, Ohio EPA; E. Osborn, ARCADIS; D. Pfeiffer, ARCADIS / Senior Scientist, Arcadis U.S., Inc. / Senior Scientist; G. Scheef, Intervet Innovation GmbH. The concentrations of hormones in surface water has received much scrutiny in recent research due to widespread detection and demonstrated effects on aquatic species at concentrations on the order of 1 ng/L. Humans and livestock are generally assumed to be the primary sources, depending upon location of study area. Wildlife, fish and plants may also contribute; however, data on the relative contributions of each group are few. To better understand the relative contribution, this study focuses on hormones originating from the excreta of all vertebrates for which there are available data: humans, livestock and representative species of wildlife and fish. The total excreted mass of two endogenously produced hormones, 17 $\beta$ -estradiol (E2) and testosterone (T) were estimated for these groups of vertebrates based on total daily excretion rates. To estimate the total mass of hormones excreted, the per capita total daily excretion mass of each hormone and the estimated total number of humans, livestock and representative species of vertebrate wildlife (fish, terrestrial herbivores, birds) were obtained. Human population statistics were obtained from 2010 U.S. census data and were stratified by sex and cycling/pregnancy status. Livestock population data for cattle, pigs and chickens were obtained from U.S. Department of Agriculture inventory statistics and were each stratified by sex and pregnancy status. The peer-reviewed literature was surveyed for information on E2 and T concentrations in excreta and total daily excretion rates. Hormone data presented as a concentration in excreta rather than a total daily excretion rate were multiplied by the estimated daily excreta production rates to obtain a daily hormone mass excreted. The total mass of E2 and T excreted by humans, livestock and wildlife are compared and the potential implications are discussed.
**661 Transport of Three Veterinary Antimicrobials from Feedlot Pens via Simulated Rainfall Runoff** S. Sura, Agriculture and Agri-Food Canada (AAFC) / Food and Bioproduct Sciences; D. Degenhardt, Alberta Innovates – Technology Futures; A.J. Cessna, National Water Research

Institute / Environment Canada, Environmental Health National Program / Agriculture and Agri-Food Canada; F.J. Larney, A. Olson, T.A. McAllister, Agriculture and Agri-Food Canada (AAFC). Veterinary antimicrobials are used in animal production, therapeutically to treat diseases and sub-therapeutically to prevent diseases and promote growth. There is a growing concern that environmental contamination by these chemicals may result in widespread bacterial resistance to antimicrobials used in human medicine. The majority of administered antimicrobials (up to 80% or more) are excreted in the feces or urine either as parent compounds or their metabolites. These antimicrobials are introduced into the environment mainly due to application of manure as fertilizer to agriculture fields or through leaching or runoff from manure storage locations (stockpiles, windrows, lagoons). Veterinary antimicrobials have been detected in manure, manure-treated soils, and surface and ground water resources near large-scale cattle feeding operations. Runoff and leaching after major precipitation events appear to be major transport pathways by which these veterinary antimicrobials eventually contaminate surface and ground water. A study was conducted at Agriculture and Agri-Food Canada Research Centre, Lethbridge, AB, Canada in spring 2011 to investigate the transport of three antimicrobials commonly used in beef cattle (*Bos taurus*) production. Beef cattle were administered the following antimicrobial treatments in the research feedlot situated on the Research Centre: (1) 44 mg of chlortetracycline kg<sup>-1</sup> feed (dry-weight), (2) 44 mg of chlortetracycline + 44 mg sulfamethazine kg<sup>-1</sup> feed, (3) 11 mg of tylosin kg<sup>-1</sup> feed and (4) no antimicrobials (control). Three pens per treatment were selected and concentrations of antimicrobials (tylosin, chlortetracycline and sulfamethazine) were measured in simulated rainfall runoff from two locations (bedding and floor areas) randomly selected in each feedlot pen. Manure and runoff samples collected from each area were extracted by accelerated solvent extraction and the extracts analyzed for chlortetracycline, sulfamethazine and tylosin using LC/MS/MS analysis. The residue data will be used to investigate the transport of the three antimicrobials in runoff and potentially generate information to support the inclusion of retention ponds in feedlots to capture runoff from feedlot pens, thereby minimizing the risk of contamination of surface and groundwater resources.

**662 Isomer-Specific Oxidation of Surfactant Metabolite Nonylphenol by Potassium Permanganate** Z. Lu; J. Gan, University of California Riverside.

Nonylphenol (NP), the metabolite of non-ionic surfactant nonylphenol ethoxylates, is one of the chemicals of emerging concern. Nonylphenol is frequently detected in soil, water and sediment due to its wide use and it is known as an environmental endocrine disruptor. Traditionally, NP is reported and studied as one compound rather than a mixture of numerous isomers. Recently, studies have shown that different NP isomers may have different estrogenic potencies and biodegradabilities in culture. However, there is no study on its isomer specific behavior during advanced oxidation processes. We investigated the reaction kinetics of 19 NP isomers (including 6 diastereoisomers) and potassium permanganate at pH 5, 7 and 9. At pH 7 with 10 mg/L KMnO<sub>4</sub> and 50  $\mu$ g/L technical nonylphenol, the half lives of 19 isomers ranged from 4.7 to 6.3 min and order of isomers with increasing half lives were: NP 119, NP 35, NP 128, NP 36, NP 37, NP 9, NP X (unknown structure), NP 38, NP 193b, NP 110b, NP 65, NP 111b, NP 110 a, NP 193 a, NP 143, NP 111a, NP 152, NP 112 and NP 194. Therefore, generally, the reaction was faster when the  $\alpha$ -position was less bulky: dimethyl>ethyl-methyl> methyl-(n or iso)-propylpentyl. This study suggested that side chain branching would significantly affect the reactivities and persistence of NP isomers during advanced oxidation processes.

**663 Organic photovoltaics: Are policies for safe disposal and recycling necessary?** Y. Zimmermann, University of Applied Sciences and Arts Northwestern Switzerland (FHNW) / School of Life Sciences; A. Schaeffer, RWTH Aachen University; P.F. Corvini, M. Lenz, University of Applied Sciences and Arts Northwestern Switzerland (FHNW). Organic photovoltaic solar cells (OPVs) are a promising renewable energy technology due to the fact that characteristics such as light-weight, semi-transparency and mechanical flexibility offer novel application possibilities. Substantial progress has been achieved during the last decade increasing power conversion efficiencies towards the crucial 10% threshold. In consideration of the anticipated forthcoming large-scale production, the environmental impact of such OPVs has to be assessed and should be compared to current best available thin film technologies. Various materials present in OPV, such as metal nanoparticles as well as fullerene-derivatives, may potentially have adverse effects in the environment. So far, no studies exist investigating a possible

release of OPV components into the environment under environmentally relevant leaching-scenarios. Whereas some research has already been carried out regarding the ecotoxicity of certain single, pure OPV components (e.g. nanoparticles), other components and/or degradation products/transformation products have not been investigated at all until now. For the first time, this study systematically composes the database about OPV materials and their fate and effects in the environment. Furthermore, priority pollutants are identified. We conclude that so far there is no evidence for an alarming risk deriving from OPVs during use phase (i.e. when intact). However, during end-of-life phase (i.e. when disposed in landfills) an increased release of harmful components seems plausible, which might result in adverse effects of OPVs in the environment. Preliminary results from our lab confirm that some compounds (organic and inorganic origin) are indeed leaching from OPVs under such conditions. By means of a variety of analytical methods such as ICP-MS, accelerated solvent extraction, SEC-HPLC and qTOF, leachates were identified. It was possible to show that inorganic compounds are actually leaching from OPV under harsh environmental conditions when applying end-of-life scenarios. These results have to be introduced into policies for safe disposal and recycling of OPVs.

**664 The influence of natural organic matter and salinity on the toxicity of nanoparticles in zebrafish embryos** G. Goss, University of Alberta / Biological Sciences; R. Clark, University of Alberta / Chemistry; D. Snell, University of Alberta; J. Veinot, University of Alberta / Dept of Chemistry; K.J. Ong, University of Alberta. The biological effects of nanoparticles on aquatic organisms have been well studied in pristine experimental media, but there is a need to determine whether nanoparticles will behave in a similar manner or have comparable toxicity in natural environmental conditions. Using single walled carbon nanotubes, silver, cadmium selenide, and zinc oxide nanomaterials, we characterize the nanoparticles in water containing natural organic matter and in a range of salinities. We find that aquatic exposure to nanoparticles in different environmental conditions can alter the biological effects of the nanoparticles, including effects on zebrafish (*Danio rerio*) embryo survival, hatch, growth, morphological development, and movement over 96 hours post-fertilization. Using both physicochemical characterization and biological effects data, we determine whether these effects are related to size, charge, composition, and/or polydispersity of the nanoparticles in different media. Furthermore, we determine the mechanism by which nanoparticles are inhibiting hatch, and determine whether the presence of humic acid abrogates these effects.

**665 Ceria(III) and ceria(IV) nanoparticles physico-chemistry in algae and daphnia exposure media and biouptake** R. Merrifield, University of Birmingham; J. Lead, University of Birmingham / School of Geography, Earth and Environmental Scien. This presentation will discuss the synthesis, characterisation and ecotoxicological effect (on freshwater algae) of ceria nanoparticles (NPs). The NPs were made in two different oxidation states (PVP-Ce<sub>2</sub>O<sub>3</sub> and PVP-CeO<sub>2</sub>) and were PVP stabilised, with similar hydrodynamic diameters of 7.5±0.6 and 6.5±0.45 nm and core sizes 4.6±0.6 and 4.2±0.8 nm, respectively. The ecotoxicological media (algae media and daphnia media) did not alter the hydrodynamic or core size (measured by STEM, FIFFF and DLS) of any of the NPs over the course of the exposure period. However the zeta potential, oxidation potential and oxidation state altered significantly for the PVP-CeO<sub>2</sub> NPs in algae media compared to no significant change in the other suspensions. The NPs were exposed to environmentally relevant aquatic freshwater algae species *chlamydomonas* using standard OECD procedures. The NPs were found to have penetrated into the algae cells and accumulated in vesicles, even at exposure concentrations of 80ppb.

**666 Role of surface chemistry of Ceria nanoparticles in organic matter interactions and in toxicity to *Caenorhabditis elegans*** B. Collin, E. Oostveen, University of Kentucky / Department of Plant and Soil Sciences; O.V. Tsyusko, University of Kentucky / Department of Plant & Soil Sciences; U. Graham, University of Kentucky / Center for Applied Energy Research; J.M. Unrine, University of Kentucky, University of Kentucky / Department of Plant and Soil Sciences, University of Kentucky / Department of Plant & Soil Sciences, University of Kentucky / Savannah River Ecology Laboratory, University of Kentucky / Department of Plant and Soil Sciences, University of Georgia / Savannah River Ecology Laboratory. Ceria nanoparticles (CeO<sub>2</sub> NPs) are increasingly being used for a variety of applications including fuel catalysts and mechanical planarization media;

however, little is known about their fate, transport and possible toxicity in the environment. To improve stability and prevent aggregation, surface coatings are commonly applied to manufactured nanoparticles but the physicochemical properties of these coatings will influence their behavior in natural systems. Once release in the environment, CeO<sub>2</sub> NPs will interact with abundant organic ligands. The adsorption of natural organic matter (NOM) to the surfaces of NPs is known to strongly influence their surface properties and aggregation behavior. Our objective is to investigate the role of NP surface chemistry and environmental modifications of NP surfaces in determining their aggregation, deposition as well as bioavailability and toxicity to soil dwelling organisms. To reach this objective, we synthesized 4 nm CeO<sub>2</sub> NPs coated with either neutral 10 kDa dextran (Dex-CeO<sub>2</sub>), negatively charged carboxymethyl-dextran (CM-CeO<sub>2</sub>) or positively charged diethylaminoethyl-dextran (DEAE-CeO<sub>2</sub>). We investigated the effects of exposure media (reconstituted moderately hard water; RMHW) and NOM on the point of zero net charge (ZPC) of particles with the various coatings at 100 mg/L. We found that the ZPC of the Dex-CeO<sub>2</sub> was reduced from around pH 7 to pH 6 in RMHW, but the addition of 100 mg/L fulvic or humic acid reduced the ZPC to less than pH 4. Neither the RMHW nor the NOM had much of an effect on the ZPC of the DEAE-CeO<sub>2</sub> particles (around pH 12) under the same conditions. The DEAE coated particles were much more toxic to *Caenorhabditis elegans* than the DEX and CM particles. Synchrotron based x-ray fluorescence imaging indicated that the DEAE particles were accumulated to a greater extent than the DEX particles. Spatially resolved XANES demonstrated that Ce in the particles in the exposure media were mainly in the +IV valence state, but up to 80 % of the Ce associated with the nematodes was in the +III valence state. This suggests that the mechanism of toxicity may involve oxidation of biomolecules coupled with reduction of Ce in the particles. We are currently investigating the role of NOM in altering the bioavailability and toxicity of CeO<sub>2</sub> NPs with various coatings.

**667 Transmission Electron Microscopy Evaluation of quantum dot uptake in fathead minnow ovarian explant cultures** C. Lavelle, Center for Environmental and Human Toxicology, University of Florida; K.J. Kroll, University of Florida / Physiological Sciences; N.D. Denslow, University of Florida / Department of Physiological Sciences and Center for Environmental and Human Toxicology, University of Florida / Dept. of Physiological Sciences &. The need for understanding the interactions of nanomaterials (NMs) with biological systems is evident, as their use in a variety of applications including cosmetics, electronics, and biomedicine is increasing. Of specific importance is the biological fate of NMs in aquatic organisms, particularly in fish, for which much is not yet understood. Previous research has shown that particle characteristics such as size and surface chemistry (including plasma protein coatings) influence biological fate and ultimately the overall effects. One class of NMs of interest are quantum dots (QD) which are structurally composed of a semiconductor core and shell that can be modified by the addition of different functional groups. The goal of this study was to determine what particle surface characteristics and physiological conditions allow for uptake of QDs into developing oocytes or their follicle cells *in vitro*. Oocytes were collected from mature female fathead minnows (FHM) and transferred to a 24-well plate with approximately even numbers of oocytes in each well. Media was supplemented with insulin (30ug/mL), hCG (10U/mL), and QDs (10 nM, ± plasma containing 17ug/mL vitellogenin). Oocytes were incubated for 24 hours and some cells were removed for confocal microscopy while the rest were preserved for histology and transmission electron microscopy (TEM). Confocal images of tissues following different treatments suggest that QDs may be taken up into oocytes/follicle cells *in vitro*. TEM analysis was used to confirm the localization of the QDs within the oocytes and follicle cells. Additionally, a separate exposure to primary cultures of follicle cells was conducted to validate QD uptake into those steroidogenic cells. Media from the follicle cell culture was assayed for the effects of QD exposure on 17β-estradiol accumulation. This assay has the potential to be a valuable tool in high-throughput screening of NMs that may cause direct toxicity to the oocyte or perturb the functions of the follicle cells.

**668 Effect of silver nanoparticles on freshwater systems: environmental feedbacks and population cycles** L.M. Stevenson, H. Dickson, University of California, Santa Barbara / Ecology, Evolution and Marine biology; E. McCauley, University of Calgary / Biological Sciences; R. Nisbet, University of California, Santa Barbara / Ecology, Evolution and Marine biology.



Engineered nanoparticles have the potential to interrupt basic ecosystem processes and can have cascading effects on entire communities. Further, ecosystem processes can, in turn, impact rates of aggregation and dissolution of nanoparticles. The availability of organic material, especially dissolved organic carbon (DOC), drastically alters the behavior and toxicity of citrate-coated silver nanoparticles (AgNPs) to freshwater phytoplankton (*Chlamydomonas reinhardtii*) and zooplankton (*Daphnia pulicaria*). In high-density algal batch cultures, greater concentrations of DOC lead to larger particle sizes and mitigate toxicity of AgNPs. Experimental removal of DOC results in disassociated particles and enhanced mortality of algal cells. At certain levels of exposure, initial toxicity to algal cultures is slowly mitigated by DOC over 48-72 hours, which allows for population recovery. Algal cultures that maintain high concentrations of DOC experience a second toxic effect, possibly due to a sustained increase in silver ion concentration. DOC also has a mitigating effect on toxicity to *Daphnia* – 100 µg/L concentrations of AgNPs cause 100% mortality in 48-hour acute tests in synthetic freshwater media but have no effect on *Daphnia* when DOC is added to the media. It is crucial to understand the mitigating effect of DOC on AgNP toxicity to freshwater algae and *Daphnia* considering the natural population dynamics of these two species. In both laboratory mesocosms and in the field, daphnid population growth may be cyclical with pronounced peaks in both *Daphnia* and algal populations. On average, algal population densities are low, but productivity remains high and the rate of DOC production by algae varies during fluctuations in algal abundance. Thus, mitigation of AgNP toxicity for *Daphnia* may be determined by algal population dynamics. To test the effects of AgNPs on interacting populations of *Daphnia* and algae, we developed a flow-through system and will present preliminary results from this design. By starting with experiments on algae and *Daphnia* alone, we are able to tease out direct and indirect effects of these particles in a dynamic system.

#### 669 Aquatic exposure models for nanomaterials based on empirical

**data** J. Quik, Wageningen University / Aquatic Ecology and Water Quality Management Group, RIVM / Laboratory for Ecological Risk Assessment; J. de Klein, Wageningen University / Aquatic Ecology and Water Quality Management Group; J. Spijker, National Institute for Public Health and the Environment (RIVM) / Laboratory for Ecological Risk Assessment (LER); D. van de Meent, Radboud University Nijmegen / Institute of Wetland and Water Research; A. Koelmans, Wageningen University, IMARES / Environment. Exposure modelling is an integral part of risk assessment of chemicals. Recent literature identified several nano-specific fate processes affecting their transformation and transport in the aquatic environment. The main removal processes are related to sedimentation and transformation processes such as changes in coating and heteroaggregation with natural colloids. This interaction with natural colloids is thought to have a dominant effect on the further fate of nanomaterials in aquatic systems. In this study we investigate different modelling approaches for the exposure concentration of nanomaterials in water. This is the first study to relate this to laboratory sedimentation measurements in natural water. In order to find the optimal model for estimating the exposure concentrations of nanomaterials in water we used three different modelling approaches ranging from simple first order removal rate constants to a mechanistical model linking homo and hetero aggregation kinetics based on the von Smoluchowski theory and stokes sedimentation theory. And third we use an empirical model based on laboratory sedimentation measurements of NPs in different natural waters. Preliminary results show that at low initial nanoparticle concentrations heteroaggregation is the main process resulting in removal of nanoparticles from the water phase. This varies with differences in the aggregate density. At low initial nanoparticle concentrations and with assumption that there is a much larger concentration of natural colloids present the removal from the water phase can be adequately modelled using first order removal rate constants. This general observation is in good agreement with the laboratory measurements. Further comparison of the model results to the dataset on CeO<sub>2</sub> nanoparticle sedimentation in natural waters resulted in estimating key parameters such as the attachment efficiency and fractal dimension of aggregates. A derivative of this model was further applied to a known water quality modelling framework, Duflow, to estimate exposure concentrations in a river catchment taking into account emissions from water treatment plants and deposition from land and air.

**670 Rapid settling of nanomaterials due to hetero-aggregation with suspended sediment** I. Velzeboer, Wageningen University; J. Quik, Wageningen University / Aquatic Ecology and Water Quality Management Group, RIVM / Laboratory for Ecological Risk Assessment; B. Koelmans, Wageningen University; D.v. Meent, RIVM. To date, sedimentation of engineered nanoparticles (ENPs) has been studied mainly in artificial media and stagnant systems mimicking natural waters. This neglects the role of turbulence and hetero-aggregation with sediment. This study aims at quantifying the removal rates of selected ENPs (CeO<sub>2</sub>, PVP-Ag and SiO<sub>2</sub>-Ag) in agitated sediment-water systems resembling fresh, estuarine and marine water types. Experimental systems and conditions were designed to mimic low energy and periodically resuspended sediment water systems (14 days), followed by a long term aging (6 months), resuspension and settling phase, as would occur in receiving shallow lakes. ENPs in systems with periodical resuspension of sediment are removed with settling rates as high as those for the sediment particles, e.g., 0.038 – 1.5 m/d, or > 1.5 m/d for marine waters. Higher settling rates of ~ 3 m/d are observed after 6 months of aging in the sediment bed at all salinities, which is explained from ENPs being progressively captured in sediment flocs. The removal rates are 1 – 2 orders of magnitude higher than those reported in recent stagnant aggregation-sedimentation studies. This implies that in dynamic natural waters, hetero-aggregation with suspended sediment is the rate determining step in removal of ENPs from the water column, and that aggregation efficiencies are close to one.

#### 671 Collaborative Monitoring and Data Sharing to Support the Whiting

**Fishery Bycatch** B. Bernstein, Consultant; D. Fraser, Whiting Fishery. In response to concerns about bycatch of four species of rockfish whose populations are severely depressed, the federal groundfish fishery management plan in 2010 implemented far reaching changes to the offshore (or mothership) fishery's management structure. These addressed the need to reduce the bycatch of key rockfish species but allowed the fishery to create its own approach to ensuring accountability at both the individual and fleet-wide levels while also spreading risk across the fishery's participants. The revised management plan, Amendment 21, created an overall total allowable catch (TAC) for the fishery and split this into catch shares assigned to individual participants based on catch history. The plan implemented a cap on the allowable bycatch and assigned a bycatch quota to each participant based on their assigned proportion of the whiting TAC. Because individual rockfish bycatch quotas were often extremely low (a fraction of a fish in many cases), fishermen ran the very real risk that a single unlucky tow could shut them down for the season. Amendment 21 allowed permit holders in the mothership sector to form coops and to create their own strategies for allocating quota and risk. All 37 participants joined a single coop and designed a system that depends on data sharing and collaborative management to spread risk while ensuring individual accountability. By combining all bycatch quota into a single shared pool, the coop created the equivalent of an insurance plan that allows fishermen to draw on the bycatch pool to cover unlucky tows as long as their overall performance is within well-defined limits. In addition to sharing data on whiting catch and rockfish bycatch, experienced skippers in the fleet have shared their combined knowledge to educate the entire fleet on methods for avoiding rockfish bycatch hotspots and understanding the topographic and oceanographic factors associated with increased bycatch risk. The fleet used this knowledge to voluntarily identify nine areas that were closed to whiting fishing. The coop manager has the authority to close areas with high bycatch, using a real time data submission and access system called SeaState. The whiting mothership sector provides a striking example of how performance standards can create the environment and incentives for the grassroots creation of a cooperative management and monitoring system that exceeds the performance of previous command and control management approaches.

#### 672 Development of Interagency Partnerships for Wetland Monitoring and Assessment: Lessons Learned from California

E.D. Stein, Southern California Coastal Water Research Project / Biology department; J. Collins, San Francisco Estuary Institute and Aquatic Science Center. Comprehensive wetland monitoring and assessment must meet the information needs of many agencies at all levels of government that are responsible for wetland protection. The challenges are to identify the common information needs, develop standardized and practicable methods to acquire the needed information, manage it effectively, and know when and why the needs are not being met. Technical challenges focus around development of assessment tools that can be used to readily convey information about function or

condition across a variety of wetland types in an accessible manner. However, even once such tools have been developed, program implementation involves unique challenges. In California, like many states, responsibility for wetland assessment and management is divided among many State and Federal agencies and programs (19 in California). Although the specific mandates and objectives vary among agencies, each requires information on extent and condition to gauge program and project effectiveness. This talk will summarize experiences over the past ten years in developing and maintaining interagency partnerships to facilitate comprehensive wetland monitoring and assessment. Collaborations often begin at the local/watershed level and have evolved to overall statewide partnerships that have been institutionalized through real-life management and regulatory decisions using a variety of formal and informal mechanisms. We will summarize lessons learned in terms of technical tool development, vertical and horizontal coordination, information management, and reporting.

**673 Initiate and Sustain Collaboration by Focusing on the Delivery of Information to the User** J. Marshack, California Water Quality Monitoring Council; K. Larsen, California State Water Resources Control Board. Government agencies, regulated dischargers and others spend millions of dollars annually monitoring California's water quality and aquatic ecosystems. Lacking an agency with over-arching authority to monitor and protect California's water resources, differing mandates have resulted in inconsistent monitoring objectives and methods to collect and assess the data, making it impossible to integrate information from multiple studies. And there is no single user-friendly place to access the information. Pursuant to state legislation and an agreement between the California Environmental Protection and Natural Resources Agencies, the California Water Quality Monitoring Council was established to address these problems. The Council published A Comprehensive Monitoring Program Strategy for California to coordinate the water quality and ecosystem monitoring, assessment, and reporting efforts of organizations both within and outside state government. Goals are to improve collective efficiency and effectiveness and ensure that resulting information is internet-available to decision makers and the public. Rather than focus on technical details, such as methods consistency and standard data formats, the Council presented a new solution – provide a platform for intuitive, streamlined access to information that directly addresses users' questions. Under Council guidance, collaborative theme-specific workgroups were formed, each developing an Internet portal focusing on one high-level question: Is our water safe to drink? Is it safe to swim in our waters? Is it safe to eat fish and shellfish from our waters? Are our aquatic ecosystems healthy? These are accessed from a single website [www.CaWaterQuality.net](http://www.CaWaterQuality.net). Portal development provides the context to effectively evaluate and resolve monitoring design, coordination, and data access problems – highlighting gaps and inconsistencies in monitoring and assessment methods and data management – within and between organizations. But funding and institutional barriers must be overcome. The legislation included no funding mechanism to initiate and sustain collaboration. State budget shortfalls and high agency workloads challenge implementation. While some agencies and organizations embrace the Council's vision, others avoid participation. In spite of the difficulties, a structure has been created to identify and prioritize actions and motivate improvement.

**674 Integrated Watershed Monitoring and Assessment using a Collaborative Approach: An Example from the San Diego Region** D. Gibson, L. Busse, B. Posthumus, San Diego Regional Water Quality Control Board; B. Bernstein, Consultant. The San Diego Regional Water Board is currently restructuring its monitoring and assessment programs from being discharger-oriented towards being waterbody-oriented. In order to successfully make this transition and realize credible and durable results, a collaborative process is often necessary to ensure that the monitoring and assessment programs are informed by a broad range of experience, expertise, and perspectives. Collaboration also helps to build support and buy-in for the monitoring and assessment programs that are ultimately developed and implemented. The goal of the project is to develop an integrated, coordinated and cost-effective watershed monitoring and assessment program for the San Diego River watershed. The integrated watershed monitoring and assessment program described here was based on a collaborative approach and reflects substantial consensus among a broadly representative group of stakeholders in the San Diego River watershed. The effort to develop an integrated watershed-scale assessment, and to improve the coordination and efficiency of monitoring and assessment includes several principles: (1) Shift attention toward

management of watersheds and/or waterbodies and away from management only of individual discharges and discharge types and their compliance with regulations, (2) Develop the ability to assess and describe the condition of beneficial uses and ecosystems rather than focusing only on simple indicators (e.g., concentration of a chemical constituent) or basic measures of management activity (e.g., numbers of inspections or 303(d) listings), (3) Implement the Surface Water Ambient Monitoring Program (SWAMP) Assessment Framework's call for question-driven monitoring that moves through a sequence of questions from impact assessment to source identification and then to evaluating solutions, (4) Ensure that permit-mandated monitoring programs are designed to support watershed scale assessment and management, and (5) Develop and maintain collaborative relationships with other entities and programs conducting related monitoring and assessments in the watershed. It is intended to use the integrative monitoring and assessment program approach in the San Diego River watershed as a prototype for analogous monitoring and assessments in other watersheds and/or water bodies in the region. The San Diego Water Board recently started an integrated monitoring and assessment program for San Diego's coastal wetlands.

**675 Site-specific Water Quality Objectives for Selenium in the Newport Bay Watershed: Habitat, Populations, and Protective Criteria** E. Byron, CH2M Hill / Environmental Services; G. Santolo, H. Ohlendorf, CH2M Hill; T.S. Presser, U.S. Geological Survey / Water Resources Division; S.N. Luoma, University of California Davis / John Muir Institute of the Environment, U.S. Geological Survey, University of California, Davis; J. Skorupa, U.S. Fish & Wildlife Service / Environmental Contaminants Div. Selenium is a naturally occurring element that readily bioaccumulates through the food chain and can cause adverse effects on higher-level aquatic life and wildlife. The Newport Bay watershed, Orange County, California, is an example where ambient concentrations of selenium exceed water quality objectives. Because of the importance of food-chain impacts from selenium, site-specific tissue-based water quality objectives (SSOs) have been proposed for selenium for the watershed and bay to be assessed by bird egg and fish tissue concentrations. The Upper Bay supports five threatened or endangered bird species: brown pelican, light-footed clapper rail, California least tern, Belding's savannah sparrow, and coastal California gnatcatcher. The protection of these listed species drove the selection of food-chain based tissue endpoints for the SSOs. Habitats are highly variable in this urban watershed. Many of the areas with highest concentrations of selenium in surfacing groundwater are low base-flow, channelized drains and often are completely concrete lined. They support almost no fish or bird use. As the drains join into the main channels they become permanently wetted and acquire more habitat of higher value. In several areas of the watershed, ponded water and marshes promote the formation of more bioavailable forms of selenium, with significantly increased ambient tissue concentrations. The lower watershed supports nesting of shorebirds and waterfowl along with reproducing populations of several species of fish. A biodynamic model was developed by USGS researchers to link the SSO tissue concentrations to waterborne concentrations. The model can be run to start with a water column concentration to predict fish and bird tissue concentrations, or, starting with fish or bird concentrations (the SSO tissue target values) to predict waterborne concentrations. Protective tissue concentrations for whole-body fish and bird eggs were chosen based on an extensive literature review and knowledge of the local, Newport Bay ecological receptor communities and habitat types. The Newport Bay watershed tissue targets, unlike standard EPA procedure, are USFWS's best estimates of true no effect values.

**676 Working toward Creative Solutions in Complex Environmental Disputes with Mediation** J.H. Reaves, Law Offices of John H. Reaves, APC. Old models produce prolonged conflicts. A broad range of disputes and claims go to litigation, which is adversarial, costly, and time-consuming. Multi-party contamination cases chew up obscene amounts of money in litigation instead of coordinating and working toward resolving the problem from the start. The developer of a project may be closed-minded to public input, inviting CEQA challenges, delays. An energy facility project may be overly wed to a siting location that sets up battles with the public. A utility may be disinterested in any public input for a large project or rate restructuring. A governmental agency's regulation or Notices of Violation may be uncompromising and tone-deaf to the burdens they cause. Each of these old models produces conflict, damages reputations, and results in great social and economic inefficiencies. What's changing? Mediation has become more

mainstream in business and tort litigation and encouraged by courts. The environmental and land use fields, however, are still rife with examples of expensive, protracted battles that may not be justified from a broader economic or social view. With current models, parties stake out early positions that harden with time, conflict, and expense. Scarce resources demand better use of funds to accomplish goals. We need to develop a more a new, more efficient model. What is mediation? Consensual. Mutual agreement as to role of mediator. Collaborative. Informal. Confidential. A way to keep the parties talking in structured, respectful manner. A way to put a human side, voice, and values to an adversary. It can preserve, restore, or build relationships. It provides close access to a neutral helper. It helps shape an efficient, coordinated game plan when desirable. It helps reassess conflict in terms of values, not positions. It allows a party to tell a mediator privately what hidden concerns exist. It provides parties "reality checks." It helps avoid or break impasses. It allows parties to address issues a court cannot. *90% of cases mediated get resolved.* What are the barriers? Following old models by habit. Assumptions about other party's motives. Negotiation strategy, including fear of appearing weak by being first to make overture. Business model of wearing party down by attrition. Ego, power, money. Regulatory self-image.

**677 Developing and Sustaining Successful Watershed Monitoring Programs: An Example from the San Gabriel and Los Angeles Rivers Watersheds, California** K. Morris, N.L. Steele, Council for Watershed Health;

S. Johnson, Aquatic Bioassay and Consulting Lab; P. Markle, S. Bishop, Los Angeles County Sanitation Districts. The San Gabriel River Regional Monitoring Program (SGRRMP) is the first comprehensive watershed-wide monitoring program in southern California. The SGRRMP was developed in 2004 by a diverse group of stakeholders representing major permittees, regulatory and management agencies, scientific and academic interests, and conservation groups. In 2007, the Los Angeles River Watershed Monitoring Program (LARWMP) was developed in the neighboring Los Angeles River Watershed using the same program design approach. The objectives of these programs are to increase awareness of the importance of issues at the watershed scale and to improve the coordination and integration of monitoring efforts to assess both compliance and ambient conditions. Monitoring commenced in 2005 and 2008 for the SGRRMP and LARWMP, respectively, and the results have already provided valuable information on the condition of surface waters at both the regional and sub-watershed scales. The success of these programs is attributed to the following factors: effective collaboration, leadership competence, stakeholder participation, communication of results, secure funding, and program responsiveness to emerging issues, policy and method developments. This presentation will provide examples of how these factors were incorporated into the design, phased- implementation, and continued operation of the programs.

**678 Undergraduate and Graduate Sampling Extends Blanchard River Watershed Partnership Monitoring** M.T Homsher, University of Findlay

/ Environmental Safety & Occupational Health Dept.; C.D. Norris, The University of Findlay / Chemistry Department; T. Albadran, A. Alghafey, G. Alzaabi, A. Abdulmalik, A. Khanal, S.J. Ramesh, University of Findlay / Environmental Safety & Occupational Health Dept.. Local university students have collaborated with the Blanchard River Watershed Partnership (BRWP) since 2005. Past activities have included Spring and Fall river cleanup by 40-60 students and faculty volunteers coordinated with the Blanchard Watershed Partnership. The Total Maximum Daily Loads (TMDL) of the Clean Water Act requires water quality and physical parameter monitoring. Students from the University of Findlay Environmental Safety and Occupational Health program initiated phosphorus, nitrate/nitrite, temperature, pH, DO, conductivity, barometric pressure, GPS coordinates, depth, width and flow rate determination. In 2011, the Blanchard River and its sub-watersheds were identified as the largest contributors of phosphorus and sediment to the Great Lakes. We responded to this concern in Ohio with harmful algal blooms, by engaging the issue with increased monitoring in Spring, Summer, and Fall 2011 with the support of a GLISTEN grant. Spring 2012 sampling and analysis was supported by The University of Findlay. Our preliminary Spring 2012 samples indicate lower loading of phosphorus, and sediment associated with less frequent rain and fewer inches of rainfall to date. Average phosphorus loadings in Summer 2011 for nine sites was 0.19 mg P/L, however sites adjacent to horse farms and failing septic systems averaged 0.27 mg P/L just below an advisory threshold of 0.30 mg P/L.

**679 Effect of activated carbon amendment and bioturbation on sediment-to-water fluxes of polychlorinated biphenyls and polycyclic aromatic hydrocarbons** D. Kupryianchyk, Wageningen University / Aquatic Ecology and Water Quality Management Group; A. Noori, M. Rakowska, T. Grotenhuis, Wageningen University; A. Koelmans, Wageningen University, IMARES / Environment. Aquatic sediments are recognized to be an ultimate sink for hydrophobic organic contaminants (HOCs) like polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs). Several processes, like diffusive transport, sediment resuspension or bioturbation, cause the release of HOCs from the sediment bed into the overlying water column. This may pose a risk to the aquatic foodweb. The contaminated mass release (flux) from the sediment bed to water interface can be described as a product of the concentration gradient between pore water and overlying water and an aqueous mass transfer coefficient,  $K_L$ . Amendment of contaminated sediment with activated carbon (AC) is meant to reduce HOC sediment-to-water fluxes. The effect of bioturbation and effectiveness of different AC treatments, however, is unknown. Moreover, it is not clear how the effects of bioturbation and AC on sediment-to-water fluxes do interfere. The aim of this study was to estimate mass transfer of PCBs and PAHs from naturally contaminated sediment that was treated in three ways: (i) capping with powdered AC (SAE Super, Norit), (ii) mixing with granular AC (1240W, Norit), and (iii) mixing with granular AC with subsequent removal of granules (sediment "stripping"). Mass transport of HOCs may be affected by biological activity in the sediment, so the effect of bioturbation by *Lumbriculus variegatus* and *Asellus aquaticus* was assessed for all three scenarios. Sediment-to-water fluxes were measured by a flux method using Empore disks as a sink for HOCs in the overlying water. For 19 PCBs studied (logKow 5.18–8.18),  $K_L$  values ranged from 0.04 to 2.5 m/d in non-bioturbated systems. The most effective AC treatment in terms of reducing PCB release from the sediment bed to overlying water was sediment capping with powdered AC, followed by sediment "stripping" with GAC and sediment mixing with GAC. Non-AC amended systems with tubificids showed apparent  $K_L$  values that were a factor of 100–200 higher than those in non-turbated systems. Biological activity of *L. variegatus* had lower effect on  $K_L$  than that of *A. aquaticus*. However, in the presence of PAC and GAC, *L. variegatus* did not cause an increase in mass transfer coefficients. This suggests that sediment amendment with AC reduced mass transfer due to strong binding of PCBs to AC, but at the same time reduced mass transfer of contaminants due to a decrease in bioturbation activity.

**680 Effect of activated carbon amendment for the treatment of parent- and alkylated-PAHs in a petroleum-impacted sediment under stagnant conditions** Y. Choi, Stanford University / Department of Civil

and Environmental Engineering, Stanford University; Y. Cho, R.G. Luthy, Stanford University / Department of Civil and Environmental Engineering. Activated carbon (AC) amendment is a promising in-situ remediation technology for the treatment of sediments contaminated with hydrophobic organic contaminants (HOCs). However, pilot-scale field studies have shown that the AC performance under field conditions may not be as readily apparent as what was observed in laboratory tests under well-mixed conditions. This is mainly because of the limited HOC mass transfer in the field during stagnant AC-sediment contact. The effect of various field conditions on AC efficacy in the field, including initial mechanical mixing, AC distribution in sediment matrices, AC particle size ranges, and pore-water movement remains poorly understood. In this study, we conducted a series of column experiments using a petroleum-impacted sediment to test the effectiveness of AC under stagnant conditions with various AC application scenarios. Our test compounds include not only parent polycyclic aromatic hydrocarbons (PAHs) but also alkylated PAHs, which are of major concern in petroleum impacted sediments. After 1 month of no-flow condition, the sediment columns amended with 75–150  $\mu\text{m}$  AC (5% dry sediment wt) with 2 minutes of initial mixing showed 60% reduction in total PAH uptake in polyethylene (PE) samplers compared to the no-AC control columns. The amount of reduction in the columns was smaller than that in a parallel test under well-mixed condition, which showed 87% reduction in PAH uptake. The observed reduction in the columns was significantly increased to 91% using a smaller AC size fraction (< 45  $\mu\text{m}$ ). When AC was applied as layers with a distance of 2 cm, the AC performance was not evident. This indicates that the distribution of AC into the sediment matrix is an important factor for the treatment performance. As sediment-AC contact time increases, the AC performance was improved. For example, for the no-flow sediment columns with 75–150  $\mu\text{m}$  AC with 2 minute mixing, the reduction increased



from 60% to 81% after 6 months of sediment-AC contact. The effect of pore-water movement was observed only for an initial short time period (i.e., one month), showing that pore-water movement is a minor factor for AC performance and becomes less important as contaminant mass transfer proceeds. The results of this study systematically show the effects of various field conditions on the effectiveness of AC amendment for the treatment of parent and alkylated PAHs.

**681 Reducing PCB bioavailability at a Canadian Brownfield site with biochar and activated carbon** B.A. Zeeb, Royal Military College / Chemistry & Chemical Engineering Dept.; M. Denyes, The Royal Military College of Canada; A. Rutter, Queen's University / School of Environmental Studies. In recent years, studies have focused on the use of activated carbon (AC) to sorb persistent organic pollutants (POPs) in aquatic sediments, and to a lesser extent in terrestrial soils. However, little data exists on the potential of using biochar for this same purpose. A preliminary greenhouse experiment, carried out in contaminated polychlorinated biphenyl (PCB)-contaminated soil (Aroclors 1254/1260), indicated that biochar added at 2.8% (by weight) to soil reduced shoot and root concentration in the known phytoextractor *Curcubita pepo* ssp. *pepo* (pumpkin) by up to 23% and 77%, respectively. Biochar also reduced the PCB concentration in *Eisenia fetida* (redworm) tissue. A subsequent field-scale study was conducted at a Canadian Brownfield site contaminated with >50 µg/g Aroclor 1254/1260 using two types of biochar added at 2.8% (by weight). In this trial, granular AC (GAC) was added at 2.8% to serve as a positive control and compare the efficiency of biochar to GAC in reducing the uptake of PCBs. Results indicate that biochar amendments significantly decreased the bioavailability of PCBs to *C. pepo* at rates comparable to GAC. Biochar also reduced PCB uptake into *E. fetida* tissue at rates not significantly different from GAC. An identical study is currently being carried out at a dichlorodiphenyltrichloroethane (DDT)-contaminated site (10.2 µg/g DDT), and results will be compared between these two contaminants. A complementary greenhouse study was conducted to evaluate the extent to which the type of mixing (i-manual and ii-soil tumbling) influences the reduction in PCB-bioavailability. Preliminary results show that reduced PCB-bioavailability does not depend on the type of mixing when the soil amendment is biochar. However, when mixing GAC into soils, reduction in PCB-bioavailability is less apparent when soil is manually mixed. Overall, these results show that biochar has potential to serve as an amendment to decrease the bioavailability of organic contaminants in the soil, and may serve as a technology for remediating Brownfield sites.

**682 Importance of black carbon on PCB sediment pore water concentrations and soluble sediment release of PCBs from Indiana Harbor and Ship Canal** A. Martinez, University of Iowa / Department of Civil & Environmental Engineering, and IIHR-Hydroscience and Engineering, The University of Iowa / Dept Civil and Environmental Eng.; C. O'Sullivan, The University of Iowa / Dept of Chemical and Biochemical Engineering; D.D. Reible, The University of Texas-Austin, University of Texas / Environmental and Water Resources, The University of Texas at Austin / Dept. of Civil & Environmental Eng.; K.C. Hornbuckle, University of Iowa / Department of Civil & Environmental Engineering, IIHR-Hydroscience and Engineering. In general, freely dissolved sediment pore water concentration of persistent organic pollutants (POPs) have been related to bioavailability, which has been shown to be reduced by adding a strong sorbent into the sediment. Here, we try to address another important aspect of freely dissolved sediment pore water concentration, soluble release of POPs from the sediment to the overlying water and how the black carbon present in the sediment affect the release. Polychlorinated biphenyls (PCBs) in bulk sediment from Indiana Harbor and Ship Canal (IHSC), a tributary of Lake Michigan, are among the highest in the Great Lakes region (median ~11000 ng g<sup>-1</sup> dw). In addition, the system also has very high values of black carbon (~30% of total organic carbon). IHSC will be dredged in the near future, and it is unclear if the dredging could decrease or increase the levels of PCBs in this system, although deeper sediments present higher PCB bulk concentrations. Freely dissolved sediment pore water concentrations of PCBs were measured using SPME PDMS-fiber in two cores and ranged from 9 to 1500 ng L<sup>-1</sup> (sum of 209 congeners as 161 peaks). These values are as 60-fold lower than estimations from organic carbon normalized partition coefficients ( $K_{PCB_i, OC}$ ). Hence, black carbon was included into a dual-carbon model which better predicted the PCB solid-water distribution coefficients. The new dual-carbon model was also used to predict PCB mass transfer coefficients

for the sediment-water flux. Our findings indicate that the release of PCBs from IHSC sediments is likely to increase after dredging but the presence of black carbon has a dampening effect on that increase: the presence of black carbon increases the mass transfer coefficient but lowers the sediment to water concentration gradient.

**684 Assessment of secondary biological responses to activated carbon amendment in Grasse River, NY: benthic community and submerged aquatic vegetation** B. Beckingham, University of Tuebingen / Civil & Environmental Engineering, University of Maryland Baltimore County / Department of Civil & Environmental Engineering, University of Tuebingen / Department of Geoscience, University of Tuebingen / Center for Applied Geoscience; U. Ghosh, University of Maryland Baltimore County / Civil & Environmental Engineering, University of Maryland Baltimore County / Department of Chemical, Biochemical, and Environmental Engineering, University of Maryland Baltimore County / Chemical, Biochemical, and Environmental Engineering. Activated carbon (AC) amendment is an innovative in situ remediation strategy for contaminated soils and sediments. One of the few field studies to date, the lower Grasse River pilot-scale AC amendment study has demonstrated in 3 years of post-application monitoring significantly reduced bioaccumulation in benthic invertebrates, reduced aqueous pore water concentrations and reduced potential for flux to the overlying water column. Importantly, in addition to understanding the variables influencing the effectiveness in reducing contaminant exposures to aquatic ecosystems, it is necessary to evaluate the other potential direct or indirect biological responses to AC amendment before this strategy can be widely adopted for sediment management. Therefore, the objectives of the present study were to assess the potential secondary effects of AC amendment to Grasse River sediments by monitoring changes in benthic community structure at field application sites, and additionally, by laboratory testing for growth effects to a submerged aquatic vegetation species, *Elodea canadensis*. The present study did not find adverse effects to benthic community structure following sediment amendment with AC in the field, yet reduced growth in *E. canadensis* was observed. The results of the test indicate that while the plants continued to grow in sediment freshly amended with AC, the growth rate was slower compared to control sediment at AC dose ≥5% dry weight. Plant growth with amendment of pine wood biochar was also significantly reduced compared to the sediment control at this dose level, which was surprising given that biochar is often cited as having beneficial effects in soil. Also interestingly, growth was reduced to a similar extent with amendment of an inert material, perlite. This demonstrates that sediment volume dilution and reduction in nutritional quality may be a key mechanism for reduced plant growth. Conclusions drawn from other published laboratory toxicity studies or field surveys have been variable and there is a need for further study into the mechanisms and factors influencing potential effects. If activated carbon amendment negatively affects ecosystem health it is clear that these effects would be organism-, system- and site-specific, and that the relative benefit of the amendment would depend on the risks posed by the contaminants present.

**685 Effects of thin-layer capping with carbonaceous materials on benthic macrofaunal communities – The other side of the coin** J. Gunnarsson, Stockholm University / Dept of Systems Ecology; G. Samuelsson, C. Raymond, Stockholm University / Dept of Systems Ecology; M. Schaanning, Norwegian Institute for Water Research / Marine Contaminants; G. Cornelissen, E. Eek, Norwegian Geotechnical Institute / Environmental Engineering. Remediation of contaminated sediments *in situ* using thin-layer capping with carbonaceous materials (CM) such as activated carbon (AC) is a recent technique that has proven successful in reducing the bioavailability and sediment-to-water transfer of hydrophobic organic compounds e.g. PCBs, PAHs and dioxins by several orders of magnitude. Thin-layer capping with CM could thus offer an efficient remediation technique, especially in larger contaminated areas, where conventional capping or dredging is not an option. A prerequisite to any remedial action for a successful environmental management is that its benefits for reducing contaminant fluxes do not concomitantly lead to a worsening of the ecological status of the ecosystem by e.g. having a long-lasting negative impact on the macrobenthic community structure and functioning. Most capping studies with CM have reported no or mild effects on benthic organisms. These studies, however, have generally been done in laboratory conditions or in severely contaminated field sediments with disturbed communities. In our studies we have tested the effects of various CM and mineral capping materials on marine coastal benthic

communities using mesocosms with undisturbed sediments and native fauna, and using field studies in the Trondheim harbour and the Grenland fjord. Structural changes in benthic communities (abundance, diversity, biomass) following capping were studied using a suite of univariate and multivariate techniques. Several biotic indices were also used for comparison of ecological status between capped and uncapped areas. Our studies show that capping with mineral materials only (e.g. clay, sand, limestone) usually have only mild and short-lived effects, whereas capping with AC have a significant negative impact on the benthic communities (ca 50 % reduction in species abundance and diversity). The severity of the effects of activated carbon also increased with time, i.e. two years after amendment. Here we summarize our major results on the effects of thin-layer capping on benthic macrofaunal communities and advocate that further ecological assessment and long-time monitoring of the effects of capping with CM on benthic communities are needed before this technique can be recommended.

**686 Effects of in-situ sorbent amendment on soil microbiology and pollutant biodegradation** D. Werner, Newcastle University / Civil Eng. and Geosciences; P. Meynet, K.M. Bushnaf, Newcastle University; R. Davenport, Newcastle University / School of Civil Engineering and Geosciences. Addition of strong sorbents to polluted soils is a novel remediation approach. To understand the long-term benefits, one needs to consider side-effects on the pollutant biodegradation. We therefore investigated the effects of 2% by weight coal-derived activated carbon (AC) in granular or powdered form (GAC or PAC) or wood-derived biochar on soil microbiology. Our results show that the pollutant biodegradation in soil is a complex function of many variables including the pollutant bioavailability which is reduced by sorbent amendments. However, the pollutant bioavailability is not always the rate-limiting factor. Batch and column studies with volatile petroleum hydrocarbons (VPHs) and sandy soils showed nutrient limited biomass growth on VPHs added as vapour or liquid phase to soil with or without biochar. Biomass yields were reduced by biochar amendment, but CO<sub>2</sub> production was comparable with and without biochar addition. The predominant bacterial community structure derived from denaturing gradient gel electrophoresis (DGGE) showed an abundance of *Pseudomonas* sp. and was differentiated in response to both, VPHs and biochar addition. Biochar amendment impacted soil microbial ecology through compound-specific changes in VPH availability and corresponding shifts in VPH consumption. In contrast, AC markedly reduced the availability of all VPHs, and CO<sub>2</sub> production was still notable, but comparably low in AC amended soil. We also investigated AC effects on the microbial community structure and functioning in outdoor lysimeter trials of polycyclic aromatic hydrocarbon (PAH) polluted soil remediation. In this instance, total microbial cell counts and respiration rates measured after 3 years were highest in GAC amended soil. The predominant bacterial community structure was not greatly affected by PAC or GAC amendment, and taxa with closest affiliations to known PAH degraders (*Mycobacterium* sp., *Rhodococcus jostii* strain RHAI, *Sphingomonas* sp., *Rhodococcus erythropolis*) remained or even increased in abundance in soil following AC amendment. We suggest that these putative PAH degraders were capable of using low concentrations of a variety of carbon substrates for their growth and maintenance, and hence were able to persist in the unamended, GAC and PAC amended soil despite very low PAH availability. The effect of biodegradation on PAH availability was most notable in unamended soil, while the lowest PAH availability overall was measured in PAC amended soil.

**687 Early Warning Program to Detect and Identify Contaminants of Emerging Concern and Their Effects to Fish and Wildlife** Z. Jorgenson, US Fish and Wildlife Service / Aquatic Toxicology Laboratory, U.S. Fish and Wildlife / Environmental Contaminants; J. Berninger, Baylor University / Department of Environmental Science; L.R. Iwanowicz, United States Geological Survey / Leetown Science Center; K. Lee, US Geological Survey; S. Choy, USFWS / Environmental Contaminants; J. Moore, Michigan State University, Wildlife Toxicology Laboratory / Dept. of Animal Science; J. Banda, D. Gefell, U.S. Fish and Wildlife Service; L.M. Thomas, US Fish and Wildlife Service / Mid-Continent Ecology Division; E. Durhan, US EPA / Mid-Continent Ecology Division, US-EPA / Research Chemist; M. Kahl, U.S. EPA / National Health and Environmental Effects Research Laboratory; P. Mazik, West Virginia Cooperative Fish and Wildlife Unit; V. Blazer, National Fish Health Research Laboratory / USGS; D.L. Villeneuve, U.S. EPA / National Health and Environmental Effects Research Laboratory, U.S. EPA; G.T. Ankley, US EPA / Office of Research and Development,

National Health and Environmental Effects Research Laboratory, Mid-Continent Ecology Division, U.S. EPA / National Health and Environmental Effects Research Laboratory. The Great Lakes basin provides an opportunity to investigate impacts to fish and wildlife from various natural and anthropogenic influences, particularly within Areas of Concern (AOC). While AOC beneficial use impairments related to chemical pollution largely encompass legacy contaminants (metals, PAHs, PCBs), many AOCs also contend with contaminants of emerging concern (CEC), such as endocrine active compounds and pharmaceuticals, where little is known about concentrations or effects. Our study explores the current concentrations of over 150 select CECs within AOCs and the potential biotic impacts to resident fish populations, naïve laboratory fish caged *in situ*, and *in vitro* bioassays. Surface water, sediment and resident fish samples were collected at multiple sites within seven AOCs. Laboratory reared fathead minnows (*Pimephales promelas*) were exposed *in situ* at five of the AOCs. Water and sediment samples were analyzed for select pharmaceuticals, hormones, and other inorganic and organic wastewater indicators. Resident fish were weighed, measured, bled and necropsied in the field. A suite of bioindicators were assessed to evaluate general and reproductive fish health. Samples from caged fathead minnows were used to both evaluate targeted endpoints associated with reproductive endocrine adverse outcome pathways (AOPs) using several apical measurements and to conduct unsupervised transcriptomic and metabolomic assessments. *In vitro* assays were conducted concurrently with the wild fish collections and the caged fish studies. Results from all analyses will be interpreted based on a variety of factors, including: geographic differences in CEC concentrations and effects in fish, the relationship between the combination of CEC concentrations and effects in fish, and possible sources of contamination. Results will contribute to our understanding of how different landscapes influence varying concentrations and composition of CECs across the Great Lakes basin along with how exposure to these compounds may affect fish and wildlife.

**688 Are endocrine active chemicals present and affecting resident fish in the Great Lakes basin? A reconnaissance study** L.R. Iwanowicz, United States Geological Survey / Leetown Science Center; V. Blazer, USGS; C. Hahn, R. Braham, West Virginia State University; H. Walsh, A. Sperry, West Virginia University Research Corporation. Contaminants of emerging concern (CECs) include endocrine active chemicals (EACs) and are commonly found in aquatic ecosystems at measureable concentrations. A multiagency project is progress in the Great Lakes basin to evaluate the significance of such chemicals. One of the many objectives of this research is to determine if CECs are present and associated with biological effects in resident fish populations. Resident fish species included in this reconnaissance study are the pelagic small- and largemouth bass, and the benthic brown bullhead and white sucker. Physiological and anatomical biomarker metrics indicative of estrogenic EDC exposure including gonadosomatic indices, gonad histology and plasma vitellogenin were evaluated. In addition, next generation sequencing technologies were employed to identify biomarker genes in these non-model species. Expression analysis was conducted to compliment physiological and microscopic pathology data as well as expression analyses of caged fathead minnows performed by others at selected sites. Nuclear receptor bioassays were used to identify the presence of EAC activity at all sample sites. Here we present biological data from the fall of 2010 and spring of 2011 field collections. Measureable vitellogenin was detected in a number of male fishes, and intersex was observed in male smallmouth bass. Nuclear receptor agonist activity was detected in grab water sample extracts from a number of sites as well. The significance of these and other findings will be discussed.

**689 Pathway level impacts of in situ exposure of fathead minnow to effluent mixtures in the Duluth harbor** N. Vinas, Mississippi State University; D.L. Villeneuve, U.S. EPA / National Health and Environmental Effects Research Laboratory, U.S. EPA; L. Escalon, US Army Engineer Research and Development; T. Habib, BTS; G.T. Ankley, US EPA / Office of Research and Development, National Health and Environmental Effects Research Laboratory, Mid-Continent Ecology Division, U.S. EPA / National Health and Environmental Effects Research Laboratory; E. Durhan, US EPA / Mid-Continent Ecology Division, US-EPA / Research Chemist; M. Kahl, U.S. EPA / National Health and Environmental Effects Research Laboratory; K. Jensen, US EPA; J. Cavallin, U.S. EPA, ORISE / National Health and Environmental Effects Research Laboratory; J. Berninger, Baylor University / Department of Environmental Science; E.J. Perkins, US Army

Engineering Research & Development / Environmental Laboratory, US Army ERDC / Environmental Processes & Effect Division, US Army Engineering Research & Development / Environmental Genomics and Genetics Team. There is a major effort to characterize the potential adverse effects of effluents released from sewage treatment plants in North America. At many locations pharmaceuticals, endocrine disruptors, and other chemicals of emerging concerns are present in the environment at concentrations capable of altering physiological processes in wildlife. In order to elucidate the effects of sewage treatment plants on wildlife, fathead minnows were caged in situ at several locations at the Duluth (MN) harbor, proximal and distal from the wastewater treatment plant discharge location. Fish were also exposed in the laboratory to 100% of effluent as a positive control. Several endpoints such as steroid hormones, vitellogenin, transcriptomics and metabolomics were analyzed. We applied a pathway level approach to understand the effects of the discharge as well as the potential dilution of the adverse effects at distal locations. Effects on gene expression were very similar for the different locations, with a higher amount of changes for the 100% effluent and the higher intensity for fish exposed to locations closer to the discharge site. Changes in gene expression were involved in pathways related to cytochrome P450s, hypoxia inducible factor, neurological function, or embryonic development among others. While distance from the discharge site decreased the effects on gene expression, pathway analysis was very effective in detecting the potential adverse effects of the discharge.

**690 Taking Metabolomics to the Field: A Pilot Study in a Great Lakes Area of Concern (AOC)** D.R. Ekman, US EPA / National Exposure Research Laboratory; D.L. Villeneuve, U.S. EPA / National Health and Environmental Effects Research Laboratory, U.S. EPA; Z. Jorgenson, US FWS; K. Lee, USGS; D. Skelton, Q. Teng, E. Durhan, K. Jensen, M. Kahl, J. Cavallin, G. Ankley, T. Collette, US EPA. Measurement of changes in endogenous metabolites via <sup>1</sup>H-NMR-based metabolomics has shown great potential for assessing organisms exposed to environmental pollutants, and thus could aid the efforts of risk assessors. However, to date, the application of metabolomics to ecological studies (i.e., eco-metabolomics) remains relatively uncommon. Rarer still is the application of eco-metabolomics outside of controlled laboratory settings, such as in the study of wild or cage-deployed organisms. With this in mind, we have been working to apply metabolomics technologies to analyze samples from organisms exposed in the field as a means to assess the health of surface waters receiving effluent from waste water treatment plants (WWTPs). Presented here are the results of a pilot metabolomics study conducted in the St. Louis River AOC, one of 43 Great Lakes AOCs. We report on the impact of exposures on the liver metabolome of caged fathead minnows (*Pimephales promelas*) at various sites, including areas receiving treated effluent from WWTPs within this AOC. These sites are characterized by complex mixtures of anthropogenic chemicals (i.e., multiple stressors) as well as other stressors characteristic of urban influenced surface waters. The impact of exposure on the metabolite profiles of the caged fathead minnows showed what appeared to be a proximity effect in which those deployed more closely to the effluent outflow responded more severely than those deployed more distally. Furthermore, the impact of exposure appeared more drastic for female fish than male fish, highlighting the importance of assessing sex dependent responses. In addition to metabolomics measurements, a variety of other molecular indicators as well as apical, whole organism endpoints have been collected from the deployed fish. Our goal is to integrate these multiple measurements in order to better track/understand fish responses resulting from exposure to the multiple stressors present in these waterways, and ultimately to assess the success of remediation efforts across multiple Great Lakes AOCs.

**691 The effect of temperature on copper kinetics in *Folsomia candida* (Collembola)** D.M. Jevtic, Jagiellonian University / Institute of Environmental Sciences; J.B. Schmidt, Roskilde University / Department of Environmental, Social and Spatial Change; N.T. Hamda, Jagiellonian University / Institute of Environmental Sciences, Jagiellonian University / Environmental Sciences; R. Laskowski, Jagiellonian University / Institute of Environmental Sciences. The importance of temperature in (eco)toxicological tests has been long recognized. Still, ecological risk assessment (ERA) protocols are based on tests conducted in one constant temperature. This approach lacks ecological realism and possibly leads to erroneous conclusions on effects of temperature in natural environments. On the other hand, understanding toxicokinetic processes and applying toxicokinetic models to the analysis of (eco)toxicity data is one of the most useful approaches in

linking the effects of toxicants over different levels of biological organization. In this study individuals of springtail *Folsomia candida* were exposed to copper-spiked OECD soil (500 mg/kg dry wt) under five different temperature regimes – two fluctuating and three constant temperatures. Fluctuating regimes represented daily fluctuations – high (from 9°C to 26.5°C) and moderate (from 15°C to 25°C). Constant temperatures corresponded to 15°C, 19.5°C and 25°C. The constant temperature of 19.5°C was chosen based on equalizing organisms' daily energy budgets with fluctuating regimes, taking into account that metabolic activity does not follow a linear relation with temperature. The time-dependent internal concentration of copper was measured at ten time points during the exposure phase (28 days) and at four time points upon transfer to an uncontaminated soil (14 days). Accumulation ( $k_a$ ) and elimination rate constants ( $k_e$ ) were estimated by fitting the time course of internal concentrations with a one compartment first-order toxicokinetic model. Our results indicate that copper kinetics in fluctuating environments follows the similar pattern compared to the corresponding constant temperature. In addition, accumulation and elimination rates were higher in 25°C compared to other tested temperature regimes, suggesting that ecotoxicological tests conducted in one single temperature may question the relevance of ERA predictions. In order to reduce the uncertainty in current ERA approaches, methods have to be revised – either by measuring toxic effects under naturalistic thermal conditions, or by developing and applying appropriate mechanistic effect models able to accurately predict toxic effects in different temperature regimes.

**692 Use of Microarrays in Linking Biological Pathways with Adverse Outcomes in fathead minnows as a result of multiple stressors across varying Landscapes** J.P. Crago, Great Lakes WATER Institute, University of Wisconsin-Milwaukee, / Student; R. Klaper, University of Wisconsin-Milwaukee / School of Freshwater Sciences, University of Wisconsin-Milwaukee / Great Lakes Water Institute. In the natural environment, fish are continually exposed to a wide variety of contaminants that can exert deleterious effects on egg production through additive or synergistic effects. Although previous studies have used microarrays to obtain expression profiles for streams with varying contaminant loads, there are few studies that tie a change in gene expression profiles to adverse reproductive outcomes, such as egg production, in the field. This study looked at 17 streams that varied in their surrounding landscape conditions and stressors. Impacts of degree of urbanization and contamination were evaluated using reproductive success in fathead minnows as an apical endpoint in conjunction with individual molecular biomarkers and microarray measurements of global gene expression patterns. The goal was to: 1) determine the impacts of multiple stressors from different landscapes on reproductive success and gene expression 2) determine whether individual molecular biomarkers or specific gene expression profiles from microarrays were associated with egg production across streams with varying landscape patterns and contaminant impacts; and 3) determine which specific water- and sediment-quality constituents were associated with the mRNA expression profiles. For the first part of the study, liver vitellogenin mRNA was shown to be useful as a short-term biomarker for egg production but its limits as a biomarker over longer periods. Microarray data revealed that there were 20 transcripts that were significantly differentially expressed in male fathead minnows in rivers where low egg production occurred. Functional analysis indicates that 12 of the 20 differentially expressed transcripts were associated with oxidative stress (8 down-regulated and 4 up-regulated). The eight down-regulated transcripts are associated with the thioredoxin pathway and selenium production. The down-regulation of the thioredoxin pathway is associated with methyl mercury, cadmium and to a lesser extent arsenic exposure. Two of the four up-regulated transcripts were associated with chromatin remodeling and DNA repair in response to cellular oxidative stress. These results indicate that oxidative stress from a variety of heavy metal exposure in these streams could play an important role in reproductive success.

**693 Comparing Effects of Complex Mixtures versus Single PCBs: Functional Outcomes of Exposure in Field and Lab Studies** M. Ottinger, University of Maryland / Department of Animal and Avian Sciences; T. Carro, University of Maryland / Animal Science; M.E. Barton, University of Maryland / Animal and Avian Sciences; L.A. Baltos, University of Maryland; K. Dean, University of Lethbridge / Neuroscience, University of Maryland / post-doctoral research fellow. Environmental exposure to endocrine disrupting chemicals (EDCs) often involves complex mixtures. Most studies have focused on acute effects, with little attention to non-lethal effects. We have



been investigating biological effects of a range of EDCs in laboratory studies on Japanese quail; embryonic exposure to a range of EDCs affected neural systems, immune response, thyroid and reproductive axes, and impaired male reproductive behavior. Recent studies have focused on polychlorinated biphenyls (PCBs) in Japanese quail and tree swallows; treatments included single congeners (PCB 126, PCB 77) and complex PCB mixtures. Eggs were injected with concentrations that bracketed those found in eggs in contaminated regions. Results showed dose dependent lethality for PCB 126 and the complex PCB mixtures. All impacted organ indices and physiological systems. However, PCB 77 did not impact lethality. This suggests that the predictive value of the Toxic Equivalency (TEQ), based on activation of the aryl hydrocarbon receptor (AhR) may not be sufficient for some compounds, such as endocrine disrupting compounds (EDCs). Other approaches have been developed to address inconsistencies in effects and to incorporate diverse data into the potency estimates. Perhaps it is time to develop a more inclusive estimation method for endocrine and neuroendocrine effects. An Endocrine Disruption Index (EDI) would complement other indices, and focus on endocrine disruption with consideration of effects beyond those mediated solely by the AhR for a comparative assessment of non-lethal EDC effects. Initial approaches in developing this index will be discussed. In summary, testing our laboratory findings in field birds has required considerations in methodologies and measurements, including field injections with continued parental incubation, consideration of environmental conditions, and assay of physiological markers of health and fitness. As such, there are some issues that must be considered when interpreting findings from laboratory and field studies, especially relative to biological actions of complex mixtures. The conclusions and opinions presented here are those of the authors, they do not represent the official position of any of the funding agencies, the Hudson River Trustees, or the United States.

**694 The estimation, confirmation, and management of risk at multiple biological scales with multiple stressors for the South River watershed.**

**Virginia USA** W.G. Landis, Western Washington University, Institute of Environmental Toxicology, Western Washington University / Institute of Environmental Tox. & Chem.; K.K. Ayre, Western Washington University; M.G. Cains, A.J. Markiewicz, H.M. Summers, J. Stinson, Western Washington University / Institute of Environmental Toxicology. We have now completed a regional risk assessment using Bayesian networks for the South River, VA. Historic industrial activities in Waynesboro, Virginia from 1929 to 1950 resulted in mercury contamination of the South River. Despite the time that has elapsed from cessation of the mercury release, Hg concentrations remain in the river, fish and wildlife. In addition to the Hg there are also additional stressors such as organic contaminants, suspended solids, nutrients, temperature and pesticides. We have calculated risk to fish, birds, amphibians and water quality from just upstream of Waynesboro to its confluence to form the Shenandoah River. The risks to organisms were calculated using levels of stressors that would affect successful reproduction of the population. The water quality parameters are based on water quality criteria and impacts to ecosystem services such as recreation. The Bayesian networks reflect causal pathways, incorporates the broad array of data and expert judgment available for the site. The networks also represent interactions at a number of biological scales. Our risk calculations demonstrate the importance of including multiple stressors, both chemical and physical and over a number for scales in order to calculate risk. While Hg is important for many endpoints, other factors such as turbidity and temperature are critical to maintaining important populations and ecosystem services. We also demonstrate how our risk assessment predictions can be confirmed by examining the patterns of risk throughout the spatial extent of the watershed and by examining different biological and ecological scales. Our results demonstrate that methods need to be developed that examine shifts in probability distributions, especially as management of toxics and nutrients are applied to the system. Subtle changes to the landscape seem to be more important than drastic alterations to only a few features.

**695 Use of Alkyl Polycyclic Aromatic Hydrocarbon Data in Evaluating the Contribution of Pavement Sealers to Urban Sediments** K. O'Reilly, J. Pietari, P. Boehm, Exponent. Environmental forensic studies have resulted in conflicting conclusions about the relative importance of pavement sealers as a source of polycyclic aromatic hydrocarbons (PAHs) in urban sediments. Published work has been based primarily on the chemistry of the priority pollutant PAHs. This limited number of constituents makes it difficult to separate similarity between sources from actual causation. Inclusion

of an extended list of alkyl PAHs and related hetero-compounds allows for more refined comparisons among sources and between sources and receptors. In this presentation, the results of a forensic investigation of urban sediments based on extended PAH data will be discussed.

**696 Role of Coal Tar-based Sealants in the Source Apportionment of PAHs in Urban Stormwater Pond Sediments** J.L. Crane, Minnesota Pollution Control Agency / Env. Analysis & Outcomes Div.. Municipalities in the Minneapolis—St. Paul, MN (Twin Cities) metropolitan area have discovered high concentrations of PAHs in some of their stormwater pond sediments prior to routine maintenance dredging. This situation can lead to more expensive disposal options when the benzo[a]pyrene (B[a]P) equivalents exceed Minnesota's human health risk-based Tier 2 Soil Reference Value (SRV) of 3 mg/kg. To address this issue, a study of surficial sediments in 15 stormwater ponds from the Twin Cities area was conducted to determine concentrations of 41 parent and carcinogenic PAHs (p-cPAHs) and 34 parent and alkylated PAHs (p-alkPAHs). Environmental forensic techniques were used to determine sources of PAHs in composite sediment samples collected from each pond. The p-alkPAH data set was used to determine mean PAH source categories as follows: 82.6% pyrogenic (combustion-related), 15.0% petrogenic (petroleum-related), and 2.4% diagenic (natural) sources. The U.S. Environmental Protection Agency's (EPA) Contaminant Mass Balance 8.2 (CMB8.2) receptor model was used to apportion sources of PAHs in sediment samples using a common subset of PAHs from the p-alkPAH and p-cPAH data sets, as well as from a separate stormwater pond study from the Twin Cities area (i.e., Met Council data set from Polta et al. 2006). For the p-alkPAHs, the model performance results were exceptionally good, and acceptable results were obtained for the other two data sets as well. The allocation of PAHs was dominated by the sum of coal tar-based sealant dust and scrapings (i.e., CT-sealant sum) for the p-alkPAH, p-cPAH, and Met Council data sets as 70.5, 67.6, and 69.4%, respectively. These results were comparable to Van Metre and Mahler's (2010) result of 72.2% CT-sealant dust from their use of the CMB8.2 model on a Twin Cities area lake. The CMB8.2 model was also run to exclude CT-sealant source profiles to further test whether CT-sealants are an important source of PAHs in Minnesota's stormwater ponds; the model supported their importance. The model results for p-alkPAHs were extrapolated based on all 34 PAHs to show CT-sealants were the most important source of PAHs (58.2%), followed by petrogenic PAHs (15.0%), and vehicle emission sources (14.7%). The p-cPAH data set was used to calculate B[a]P equivalents two ways, including using proposed EPA relative potency factors, and the results were compared to the Tier 1 and 2 SRVs to evaluate disposal options for dredged material from these ponds.

**697 Acute Toxicity of Runoff from Coal-Tar- and Asphalt-Based Pavement Sealants to Two Aquatic Organisms** B. Mahler, U.S. Geological Survey / Water Resources Discipline; P. Van Metre, U. S. Geological Survey; C.G. Ingersoll, USGS / Columbia Environmental Research Center, U.S. Geological Survey / Columbia Environmental Research Center; J.L. Kunz, USGS, US Geological Survey / Department of Toxicology. There are anecdotal reports that runoff from pavement with newly applied coal-tar-based (CT) sealcoat is toxic to aquatic life; no information is available regarding toxicity associated with runoff from pavement with asphalt-based (AS) sealcoat. We measured chemistry and toxicity of runoff collected periodically from 5 hours to 36 days after application of CT and AS sealcoat; runoff from unsealed pavement was collected once. Sites were at the University of Texas, Austin, Texas. Unfiltered runoff was analyzed for 27 polycyclic aromatic hydrocarbons (PAHs). Standard toxicity tests were done with a cladoceran (*Ceriodaphnia dubia*) and fathead minnows (*Pimephales promelas*) using 48-hour exposure to undiluted and diluted (1 part runoff from sealed pavement to 9 parts control water) runoff under ambient laboratory lighting; 24-hour survival of organisms following a 4-hour pulse of ultraviolet radiation (UVR) also was evaluated. Concentrations of PAHs in runoff from CT-sealcoated pavement (CT runoff), on average, were about 10 times higher than those for runoff from AS-sealcoated pavement (AS runoff). Concentrations in runoff from the unsealed pavement were near or less than the detection limit and non-toxic to test organisms. In the CT-runoff treatment, there was 100% mortality (both species) within 24 hours of exposure to undiluted CT runoff collected 4, 12, 24, 48, 72 hours and 7 days following application. There was no mortality with the 10% dilution of CT runoff without UVR, but with UVR there was 100% mortality of *Ceriodaphnia dubia* for as long as 64 days following sealcoat application.

Mortality of organisms exposed to undiluted AS runoff occurred only following the pulse of UVR (to Day 36 for *C. dubia* or 48 hours for *P. promelas*). The results demonstrate that PAH concentrations and toxicity associated with CT runoff greatly exceed those associated with AS runoff, and that toxicity continues at least several weeks following application.

**698 PAH Source Characterization using Q-Mode Eigen Methods** R.W. Chappell, Environmental Science Solutions LLC; R.L. Olsen, CDM

Smith. Forensic investigations to characterize sources of polycyclic aromatic hydrocarbon (PAH) compounds, which may be introduced into the environment through diverse and complex natural and anthropogenic processes and pathways, generally involve identifying and evaluating corresponding multivariate profile patterns. One group of multivariate statistical methods, broadly referred to as Q-mode eigen methods, endeavor to simplify source characterization by mapping PAH profile similarities in reduced multi-dimensional space, thus providing a framework to qualitatively evaluate, distinguish between, and otherwise fingerprint possibly multiple different PAH sources. This paper demonstrates various Q-mode methods and their application to PAH concentrations in soils at a former chemical site in the northeastern United States. At issue was characterizing the degree to which PAH concentrations may be attributed to fugitive site sources versus urban or ambient sources. The paper also discusses important data treatment issues prior to multivariate analysis, such as the handling of missing data and nondetects.

**699 A Risk-Based Remedy for Wood Treating Compounds in Estuarine River and Marsh Sediments adjacent to the Former Gautier Oil Site,**

**Gautier, MS** T. Iannuzzi, J. Beckner, L. Fontenot, ARCADIS; M. Adkins, CSX Transportation, Inc.. The former Gautier Oil Site, Gautier, MS, was used for wood preserving (creosote treated piles, bridge timbers, and cross-ties) operations for more than a century, affecting on-site areas and sediments in adjacent waterways. A remedy was implemented to contain and recover free product wood treating compounds on-site. A risk assessment and feasibility study (FS) were performed for the offsite estuarine areas, including Bayou Pierre (marsh) and the West Pascagoula River. The key issues were contaminated sediments and bioavailability and potential toxicity of polycyclic aromatic hydrocarbons (PAHs). The risk assessment was focused on potential direct and indirect impacts of PAHs to the estuarine food web, and human exposures via direct sediment contact and ingestion of local seafood. A range of sediment and biological monitoring data were collected to support the risk assessment. Human health and wildlife risks (via food web exposure) from PAHs were determined to be *de minimis* based on these data. A sediment quality triad (SQT) assessment was conducted to quantify risk associated with impacted sediments to benthic communities in the in the aquatic/wetland areas immediately adjacent to the site. The SQT, in conjunction with a thorough understanding of the ecology and fate/transport processes at the site, was used to help define potential sediment remediation needs and options. The results suggest that the benthic invertebrate communities in the River and Bayou adjacent to the site are similar in diversity, abundance and structure to those in reference locations, despite variable and elevated levels of PAHs in near-site surface sediments. Site-specific toxicity was limited—an ecological effects range of 216 to 826 mg/kg total PAH was developed for benthic community impacts. This range is comparable to findings from similar studies at other wood treatment facilities. From these data, a conservative remediation goal of 200 mg/kg was selected for surface sediments adjacent to the site. The RI/FS and risk assessment results were used to define the impacted areas to be remediated and to help develop a focused remedy for the sediments at the site that would reduce exposure, and that would foster future attenuation of PAHs in sediments. To mitigate for potential impacts of the remedy on wetlands habitats adjacent to the site, an ecological restoration program was developed and implemented to provide enhanced habitat within the River and Bayou system.

**700 Sediment Contamination in the Gowanus Canal: Establishing the Current Sources of PAHs using Multiple Lines of Evidence** E.A. Garvey,

Louis Berger Group, The Louis Berger Group, Inc. / Site Assessment and Remediation; S. Gbondo-Tugbawa, C. Prabhu, S. McDonald, The Louis Berger Group, Inc.; E. Mahoney, New York City Department of Environmental Protection. The Gowanus Canal in Brooklyn, NY has become a center of environmental interest as the City of New York, the USEPA and the National Grid Corporation evaluate the extensive contamination that lies within its banks. The Gowanus Canal itself is a small, urbanized, largely

commercial waterway with extensively bulkheaded shorelines. Its use for commercial shipping extends back to the late 19<sup>th</sup> century, and parallels the growth and decline of industrial activities in Brooklyn. There are a number of industrial contaminant sources to the canal, in particular, three manufactured gas plants operated along its banks during the first half of the 20<sup>th</sup> century. Additionally, several fuel storage facilities operated along its banks as well. CSO discharges also impact water quality in the Canal. Several investigations conducted by the USEPA and National Grid Corporation provide data to document the extent of contamination in the Canal and the immediate upland areas. Limited data are also available to describe the chemical loading from the CSO discharges. EPA anticipates issuing a ROD in the near future, including a possible ecological PRG for PAHs that may be used as a basis to regulate CSO discharges to the Canal. This presentation will describe the current nature and extent of contamination in the Canal with a focus on PAH contamination. It will also describe the City's working conceptual site model for the site, including the importance of current groundwater loads, the extent of sediment contamination, and the role of CSO discharges in determining PAH levels for both existing conditions and potential future conditions. The historical uses of the Canal have resulted in extensive levels of PAH compounds and oil residues in the sediment. The unique history of industrial discharge and residential groundwater usage in the vicinity of the Canal has led to the migration of PAH-bearing NAPL beyond the estuarine sediments lining the Canal and into the native soils below. An important component in the analysis of current conditions is the use of geochemical evidence to determine an initial solids balance and preliminary estimates of relative contaminant loads to the Canal. The geochemical evidence suggests the dominance of harbor solids in the sediments accumulating within the Canal. Additionally, anticipated improvements resulting from CSO upgrades currently under construction will be described as part of the future conditions.

**701 Effect of phenanthrene and/or pyrene on metabolomic profiles in the Japanese medaka (*Oryzias latipes*)** S. Uno, Kagoshima University / Faculty of Fisheries; K. Nakayama, Ehime University / Center for Marine Environmental Studies; E. KOKUSHI, Kagoshima University / Faculty of Fisheries; A. Shintoyo, K. Ito, Kagoshima University; J. Koyama, Kagoshima University / Faculty of Fisheries. The effects of phenanthrene and/or pyrene on metabolomics profiles in the Japanese medaka (*Oryzias latipes*) were examined using GC/MS. Phenanthrene and pyrene are common polycyclic aromatic hydrocarbons (PAHs) generally release by hydrocarbon combustion or oil spill. Some PAHs are known to cause deformities or growth retardation in fish embryos and juveniles. However, little is known about their combined effects. All exposures were performed on one month old medaka and fish were collected at 0, 24, and 96 hr without feeding. Metabolites with significantly different levels between exposed and control groups were screened by one-way of variance (ANOVA). Principal component analysis (PCA) was then applied to the screened metabolites data, and principal component (PC) scores for each sample obtained. Each individual exposure group of phenanthrene and pyrene clustered at similar place on two dimensional PCA score plots, being separated from control group along the PC1 axis. This result suggests that the effects of individual exposure of phenanthrene and pyrene produced similar metabolite profiles in medaka. In contrast, the group exposed to phenanthrene and pyrene together clustered away from control group along the PC2 axis. This suggests that the combined exposure to both PAHs induced different effects to medaka than induced by exposure to individual PAHs. The differences in metabolite profiles between individual and combined exposure groups were due to variations of amino acids and glucose. All essential amino acids in the individual exposure groups increased from 0 to 24 hr, while those in control were decreased over the same period. These increases in were likely caused by degradation of the proteins in medaka muscle generating essential amino acids needed to provide the excess energy needed to eliminate phenanthrene or pyrene. On the other hand, those in the group exposed to both of phenanthrene and pyrene behaved similarly to the control group from 0 to 24 hr, seemingly unaffected by the combined PAH exposure. However all essential amino acids and glucose in the combined exposure group didn't change between 24 to 96 hr, while amino acids in the control significantly increased over this time period. These results suggest that the combined exposure of phenanthrene and pyrene inhibited protein degradation in medaka muscle and could not generate the excess energy. The effects by combined PAH exposure were unpredictable from the individual exposure.



**702 Fully automated, trace level detection of PAHs in saltwater by online-SPE LC-MSMS: the quest to eliminate sample preparation** C. Ramirez, Florida International University; C. Wang, Florida International University / Department of Chemistry & Biochemistry; P.R. Gardinali, Florida International University / Department of Chemistry & Biochemistry and SERC. Along with volatile aromatic hydrocarbons, PAHs are considered one of the major contributors to the toxicity of crude oils. Following a release of crude oil or petroleum distillates in environmental waters PAHs are often used as markers to determine not only the extent of contamination and fate of the released product but also the potential effects of the spilled oil on natural resources. Therefore, there is a clear need for fast, reproducible and sensitive methods to quantify PAHs in large numbers of samples in a short period of time. GC-MS and HPLC-FLD have been extensively used to isolate and quantify these compounds. GC-MS combines labor-intensive extraction and cleanup steps, and HPLC-FLD lacks the required resolution and selectivity to address coelutions. In order to overcome these challenges, this study uses a dopant-assisted atmospheric pressure photoionization tandem mass spectrometry (APPI-MS/MS) method combined with online SPE followed by UPLC separation to provide a one-step protocol to detect PAHs at low part-per trillion level with very limited sample preparation. Seawater samples were fortified with isotopically labeled PAHs and loaded to a Hypersil Gold-aQ column (20x2.1 mm, 12  $\mu$ m), using an auxiliary pump for sample preconcentration and salt removal. The SPE column was then connected to an UPLC pump and backflushed to a Hypersil Green PAH analytical column (150x2.1 mm, 3  $\mu$ m) where a 20 min gradient separation was performed at a flow rate of 0.600 mL/min. The gas-phase dopant (Chlorobenzene) was delivered directly into the APPI source. Detection was performed by a TSQ-Quantum Access QqQ Mass Spectrometer equipped with a Syagen Photomate source (10.6 eV Kr lamp). The automated system performs the injection, online preconcentration, seawater matrix removal, UPLC separation and selective MS/MS detection in 28 min. SRM was used to detect parent and alkylated PAHs, including all target analytes listed by the USEPA and the EU as priority pollutants. Calibrations were linear ( $R^2 > 0.99$ ) in the ranges tested (5 to 500 ng/L for 10 mL injections), method detection limits (MDLs) ranged from 6 to 21 ng/L in fortified seawater (sample size 10mL), and analysis of standard reference materials yielded recoveries between 85 to 117%. The full method is comparable to traditional GC/MS and, because of the absence of sample preparation, is particularly advantageous for the real time analysis of single compounds during short or long-term toxicological studies.

**703 Applications of Spatially-Explicit Bioaccumulation Modeling** K. von Stackelberg, E Risk Sciences, LLP. Bioaccumulation of sediment-associated contaminants may lead to potential exposures and risks in ecological and human receptors consuming fish. This presentation describes several applications of a spatially-explicit bioaccumulation model, *FishRand*, which offers the ability to incorporate spatial and temporal aspects of exposure by simulating fish migration, foraging area, and site use, which can be important drivers of actual exposures. The probabilistic framework allows for predictions of population distributions for direct estimates of risk to fish. Alternatively, for estimating risks to fish-consuming ecological and human receptors, the model provides additional uncertainty distributions. In addition, the probabilistic framework allows for greater linkages with other models such as economic models that cannot use deterministic results, as well as decision analytic frameworks.

**704 Refinement of pesticide exposure assessment for birds to include spatial and temporal variation: a modeling approach** T. Kulakowski, University of Reading / School of Biological Sciences; K.A. Kulakowska, University of Reading / School of Biological Sciences; R. Sibby, University of Reading. Agent-based population modeling is a promising way to develop ecological risk assessment towards increased ecological realism. Pesticide exposure of wild living animals is heavily influenced by their interactions with changing environment. Incorporation of landscape structure and dynamics as well as species-specific ecology in spatially explicit population models enables better estimation of the risks pesticides pose to wildlife. We have developed a population agent-based framework and implemented models of two bird species: skylark (*Alauda arvensis*) and woodpigeon (*Columba palumbus*). We chose them because of their importance in risk assessment: skylarks nest in arable fields and so are at risk from pesticides throughout the breeding cycle, while woodpigeon are at risk because they eat the leaves and seeds of arable crops. Furthermore, they represent two different groups of

birds: *Alaudidae* and *Columbidae*. This enables more flexibility in analyzing impacts of different behavior on exposure patterns. For both species there is abundant ecological data available for model parameterization and testing. Except of ecological literature we used two extensive long-term studies conducted in the UK. Woodpigeons were observed between 1960 and 2005 and skylarks between 2000 and 2010. The model framework is designed to enable quick implementation of additional bird species with as little effort as possible. The core of birds energetics and breeding, landscape management and data analysis is common for all the species, while specific behavior for each species is an extension to the model. The model simulates realistic landscape using GIS data. Here we briefly present the model design and evaluation. In addition we describe a case study where we compare exposure of modeled populations to different pesticide application scenarios. We compare pesticide application on winter wheat and oilseed rape in two different times in spring corresponding to different stages of breeding cycle of modeled species. We conclude that spatial structure of landscape together with species ecology can affect exposure in a way that is difficult to predict without modeling. In this way we show that including realistic landscape and species specific behavior is important in pesticide exposure assessment.

**705 Application of a spatially explicit exposure model to inform remedial decision-making at a Superfund Site** J. Isanhardt, R. Blackburn, S. Skipper, U.S. Fish and Wildlife Service; D. Wall, USEPA. Ecological risk assessment is a critical and complex component of the Superfund decision-making process. Often ecological scenarios are overly simplified or not evaluated properly due to costs or schedule. Many Superfund sites do not have the budget or schedule to properly assess the complex exposure variables such as receptor life history traits and other ecological processes. The use of a spatially explicit exposure wildlife model provides an additional tool toward a more scientifically defensible decision-making process in ecological risk assessment. Use of the Spatially Explicit Exposure Model (SEEM) incorporates habitat suitability and foraging habits in order to better estimate wildlife exposure at contaminated sites. Also, rather than assess risk for a single representative individual, SEEM tracks the foraging behavior of all individuals in a local population, thereby providing population-level estimates of risk that can be interpreted by natural resource and risk managers. Exposure models employ several site-specific variables (e.g., habitat types, foraging range, population size), which likely improve the assumptions associated with exposure point concentrations used in ecological risk assessment; however the accuracy of model results are not always evaluated. We conducted biological monitoring at Eureka Mills Superfund Site, Eureka, Utah, where lead contamination is an issue, to assess whether breeding songbirds that appear to be most at risk as predicted by SEEM are being exposed to elevated lead levels. To address the potential for adverse effects from lead exposure in breeding songbirds, we compared results of traditional deterministic exposure assessments with a spatial model and compared the results of both with blood-lead concentrations from songbird species at Eureka Mills, a site impacted by historic mining wastes. The results from this study can be used to (1) prioritize areas within the study area for cleanup relative to terrestrial receptor use if adverse effects or the potential for adverse effects are detected; (2) provide a further understanding of the strengths and weaknesses associated with spatially explicit exposure modeling techniques; and (3) clarify interim benchmark concentrations (i.e., potential cleanup and monitoring levels).

**706 Kicking the tires: corroboration of model output with field data of the spatially-explicit exposure model (SEEM)** M. Quinn, US Army Institute for Public Health / Health Effects Research Program, US Army Public Health Command / Health Effects Research Program; M.S. Johnson, US Army, Public Health Command / Health Effects Research Program, US Army Institute of Public Health / Health Effects Research Program, Army Institute of Public Health / Health Effects Research Program, US Army Ctr for Health Promotion & Preventive Med. / Health Effects Research Program; T. Wickwire, Exponent Inc.. Understanding the influence of habitat and contaminants in soil on a spatial scale is critical to predicting exposure patterns. Current practice often simplifies these associations through the use of conservative assumptions that are rarely predictive. We tested the Spatially Explicit Exposure Model (SEEM) at two skeet ranges and investigated risk of lead (Pb) exposure to small mammalian species. Habitat was classified into three general categories (high, medium, and low) as were soil Pb concentrations. Mean daily exposure estimates for populations of small rodents were modeled and compared with the EcoSSL for Pb to calculate a



risk estimate. These estimates were compared with blood Pb concentrations of these species at each site with the resulting risk estimated derived through the use of a tissue-specific toxicity reference value. This effort illustrates the benefits and shortcomings of using SEEM at contaminated sites and provides evidence toward greater use in making informed land-use decisions.

**707 Using spatially explicit data, geochemistry and modeling to inform ecological risk assessment for a proposed pit mine expansion** D.A. Rudnick, L. Williams, Integral Consulting Inc.; J. Ortega, Newmont Mining Corporation; A. Nicholson, M. Aldea, Integral Consulting Inc.. A screening level ecological risk assessment (SLERA) was conducted for a proposed Pit Lake, an expansion of an existing Pit Mine owned and operated by Newmont Mining Corporation in the arid Great Basin ecosystem of north-western Nevada. As is typical for SLERAs, conservative assumptions for both toxicity and exposure to the ecological community were initially used to evaluate the potential for unacceptable risk. As a result, the initial screening approach provided minimal information to refine the list of chemicals of interest needed to focus risk management and reduction strategies. To provide a more complete evaluation of potential risk, spatially explicit modeled and empirical site data was incorporated into the assessment for multiple media including soil and expected future sediment and surface water conditions over multiple time periods. These data were used in conjunction with an expanded list of toxicity values and exposure pathway models to evaluate risks to ecological receptors at the site. Multiple exposure scenarios were used to evaluate both baseline risks. The results of exposure modeling indicated that a handful of metals, including aluminum, antimony, arsenic and mercury, showed potential for risk to some ecological receptors. However, an evaluation of geochemical conditions helped to further inform the risk assessment by showing that expected conditions at the future Pit Lake are likely to limit bioavailability to ecological receptors, reducing the potential for unacceptable risk. Opportunities for risk mitigation were explored by evaluating whether surface materials with lower chemical concentrations could be placed at the site in order to reduce exposure to areas with higher levels of chemicals of interest. The results of this assessment illustrates the importance of a robust, spatially and temporally explicit data set, an in-depth understanding of site-specific geochemistry, and consideration of site management alternatives that may be available, in order to develop an accurate understanding of the potential for unacceptable ecological risk at mining sites.

**708 Expanding the utility of spatially explicit exposure modeling: applications beyond site-specific assessments** C.J. Salice, Texas Tech University / Environmental Toxicology, Texas Tech University / TIEHH, Texas Tech University / Department of Environmental Toxicology. Spatially explicit ecological risk assessments represent a marked improvement in the risk assessment process by moving away from restrictive assumptions about exposure. Generally, spatially explicit modeling is most suited to site-specific assessment. However, the spatially explicit approaches can be applied to a broader range of risk assessment scenarios including national-level assessment for pesticides and for exploring potential management or clean-up scenarios. I use examples from two different assessments in which spatially explicit concepts and models were used to generate improved estimates of risk in the one case and to explore clean-up strategies in another. In the first example, an important assumption in many risk assessments is that receptors occupy contaminated habitats 100% of the time and subsequently consume 100% contaminated diet at an upper-end contamination level level (e.g. 95<sup>th</sup> percentile). Alternatively, incorporating foraging behavior and the variability of residues on food items is an indirect way of accounting for spatial heterogeneity. In the simplest approach, receptors average exposures by foraging over a broad area and, hence, are assumed to receive the mean residue exposure. But because risk endpoints are frequently thresholds, variability in food item residues has important implications for exposure and risk estimates. For birds foraging in pesticide treated fields for example, including spatial variation in residue concentrations on food items results in similar mean exposure levels compared to just assuming birds always consume mean residue food items but some birds will receive a higher maximum exposure and hence, the actual estimates of risk are vastly different. In a second example involving a population-level assessment of mink inhabiting a PCB contaminated river, PCBs were modeled as distributed heterogeneously with hot spots of contamination of varying degrees. Simulations of remediation strategies indicated that a focused clean-up on hot spots proved very effective although the effectiveness was impacted by how quickly clean-up was initiated after contamination and the percentage removal of PCBs. Nonetheless,

the model pointed to the importance of spatially explicit data and management options. These examples illustrate the utility in using spatially explicit approaches for risk assessment and environmental management even under circumstances where site-specific data may be lacking, sparse or non-existent.

**709 Lessons learned by comparing results of spatial models to non-spatial assessments** R.T. Rytí, Neptune and Company, Inc.. Watershed-scale ecological risk assessments for the Los Alamos National Laboratory (LANL) in New Mexico, USA have utilized complex spatial models to assess wildlife exposure. For assessments of individual contaminated sites a simple screening level approach is typically used. In some cases the simple methods are followed by more complex assessments. This paper contrasts the results of the non-spatial assessments with those provided by the ECORISK model, which was developed for use at LANL. ECORISK integrates biological, ecological, and toxicological information using geographic information system interfaces so that model input and output are spatially explicit on the grid system basis. When comparing the spatial and non-spatial model results one should consider what methods were used to calculate representative soil concentrations, the granularity of the assessment based on the model structure, and the spatial boundaries evaluated in the assessments. One advantage of the ECORISK model results is that they can be expressed as probabilities of adverse effects. In contrast the non-spatial assessments simply provide qualitative information on the likelihood of adverse effects. ECORISK model results for an endangered species, the Mexican spotted owl, based on exposure to contaminants in soil and modeled uptake into their prey are used to illustrate these points.

**710 Spatially Explicit Wildlife Exposure Models (PANEL): Moving Toward Their Increased Acceptance and Use** M.S. Johnson, US Army, Public Health Command / Health Effects Research Program, US Army Institute of Public Health / Health Effects Research Program, Army Institute of Public Health / Health Effects Research Program, US Army Ctr for Health Promotion & Preventive Med. / Health Effects Research Program; B. Hope, CH2MHILL; T. Wickwire, Exponent Inc.. Limitations inherent in models currently used in ecological risk assessment have spurred demand for spatially explicit exposure models. These models are scientifically sound and technically innovative tools for more realistically capturing potential wildlife exposures to chemicals in the environment. Currently available spatially explicit exposure models are diverse and powerful tools for expanding analytical capabilities, improving risk estimates, communicating these estimates to a broad audience, and creating more effective risk management outcomes. Their use has thus far been limited by a lack of information about available models and their capabilities, along with few examples of how they may be applied in actual assessments. This current lack of widespread use should not, however, be interpreted as implying that spatially explicit exposure models are not valuable tools in the risk assessment toolbox. The overall objective of this session is to emphasize the value of spatially explicit exposure models, encourage their increased use, and illustrate how they can provide more realistic estimates of risk to mobile ecological receptors. Using case studies when applicable, presentations in this session will identify impediments to use/acceptance of spatially explicit exposure models, how model outputs fit into the classic ecological risk assessment framework, and how these outputs provide value to the risk management process. Finally, a path for moving spatially explicit exposure models into the mainstream of ecological risk assessment will be discussed.

**711 In ovo toxicity of methylmercury in thick-billed murre and arctic terns** B.M. Braune, A.M. Scheuhammer, D. Crump, S. Jones, D. Bond, Environment Canada. Mercury has been increasing in some marine birds in the Canadian Arctic over the past several decades. To evaluate the potential reproductive impact of mercury exposure, eggs of two species of arctic-breeding seabirds, the thick-billed murre and arctic tern, were dosed with graded concentrations of methylmercury and artificially incubated in the laboratory to determine species differences in sensitivity. Based on the dose-response curves, the median lethal concentrations (LC<sub>50</sub>) for thick-billed murre and arctic tern embryos were 0.48 mg g<sup>-1</sup> and 0.95 mg g<sup>-1</sup> mercury on a wet-weight (ww) basis, respectively. Compared with published LC<sub>50</sub> values for other avian species, the murre and terns had a medium sensitivity to methylmercury exposure. LC<sub>50</sub> values were also calculated for the actual mercury concentration measured in the embryos, that is, the maternally-deposited mercury plus the injected methylmercury dose. This increased the LC<sub>50</sub> values to 0.56 mg g<sup>-1</sup> mercury ww in the thick-billed murre and to

1.10 mg g<sup>-1</sup> mercury ww in the arctic tern. The incidence of gross external anatomical deformities was 4.3% in the murre embryos and 3.6% in the tern embryos. However, given that the eggs were taken from wild populations, it is unlikely that the deformities observed in this study were due to methylmercury exposure alone.

#### **712 How much mercury is too much: dosing and field studies on songbirds**

**D.A. Cristol**, College of William & Mary / Professor of Biology, College of William & Mary / Department of Biology, College of William & Mary / Associate Professor, College of William & Mary / Dept. of Biology. Multi-year field studies on tree swallows, Carolina wrens and eastern bluebirds have been combined with a year-long dosing study on zebra finches and European starlings to produce a more comprehensive assessment of the sub-lethal effects of dietary mercury on songbirds. The experimental dosing studies have confirmed the effects of mercury on behavior. Mercury affected singing behavior in both captivity and the field, and in captivity additional cognitive effects were detectable. Effects of dietary mercury on reproductive success were also evident in the field and mercury's causative role was confirmed through dosing studies. Reproductive success was reduced at relatively low levels of mercury, but some birds maintained the capacity to breed successfully at much higher levels of mercury intake than others. The relatively unchallenging life of captive birds allowed them to reproduce successfully at levels of mercury that were predicted to have been catastrophic in the field. Analysis of endocrine endpoints – testosterone, corticosterone and thyroid hormones – have not yet confirmed the results of field studies that suggested endocrine disruption by mercury. However, careful study of the immune system in captive dosed birds, in particular reduced or delayed proliferation of B-cells, was consistent with the finding of immune suppression from field studies. The experimental manipulation possible in captivity provides rigorous evidence that mercury reduces fitness in songbirds while field studies offer guidance as to the levels of mercury that actually cause biological harm under natural conditions.

#### **713 Neurochemical biomarkers to assess mercury risks in wild birds: potential applications, thresholds, and limitations**

**J. Rutkiewicz**, University of Michigan; N. Basu, University of Michigan / Department of Environmental Health Sciences. Mercury (Hg) is a neurotoxic metal known to accumulate in tissues of wild birds. Studies have identified Hg associated brain lesions, behavioral changes, and reproductive impacts in several avian species. These endpoints are vital for risk assessment but their predictive value is limited. Neurochemical biomarkers, biochemical changes that occur prior to overt damage, may represent subclinical markers of early effects and may help overcome some limitations associated with apical endpoints. Here we review studies by us and others aiming to derive Hg-associated neurochemical thresholds and acknowledge pros/cons to this approach. In the field, a study of bald eagles and common loons with high Hg exposure across Canada found associations between brain Hg and muscarinic cholinergic and N-methyl-D-aspartic acid (NMDA) receptor levels. A study of eagles with comparable exposure across the US Great Lakes found similar associations between brain Hg and the NMDA receptor and the enzymes glutamic acid decarboxylase and glutamine synthetase. Conversely, studies of herring gulls and common loons with low Hg exposure found no associations between brain Hg and neurochemical receptors, thus suggesting a threshold (5-10 ppm Hg in brain) for neurological change exists. Relating Hg burdens in wild birds to neurochemistry may help determine toxic thresholds for risk assessment. However, potential drawbacks and limitations to neurochemical biomarker use exist. A study of postmortem stability of several biomarkers reveals that many are stable under various environmental conditions but others are less stable and their utility may be limited to freshly collected samples. Impacts likely vary across developmental stage. Recent Hg air cell egg injection techniques have been applied in an attempt to validate biomarkers and identify thresholds for change in embryos and hatchlings. Despite accumulating high levels of brain Hg, chicken, quail, and murre embryos did not consistently display the Hg associated neurochemical associations seen in wild birds. These studies underscore the great variability of responses between species, age classes, and exposure routes. Nevertheless, neurochemical biomarkers may have useful applications for assessing Hg risks in wildlife and help to expand our overall understanding of the mechanisms of Hg toxicity.

**714 Effect of methylmercury on foraging behavior of larval yellow perch (*Perca flavescens*) and zebrafish (*Danio rerio*): implications for recruitment** **F.X. Mora**, M. Carvan, University of Wisconsin – Milwaukee; C.A. Murphy, A.D. Deardorff, Michigan State University / Department of Fisheries and Wildlife; N. Basu, University of Michigan / Department of Environmental Health Sciences; J.A. Head, National Oceanic and Atmospheric Administration; F.W. Goetz, University of Wisconsin – Milwaukee. Yellow perch (*Perca flavescens*) is a fish species of economic and ecological importance in the Great Lakes. In Lake Michigan, this species has faced difficulties with successful recruitment. Low recruitment has been widely attributed to overfishing, however very few studies have explored the effect of contaminants, specifically methylmercury, on larval yellow perch. Methylmercury is environmentally present in the Great Lakes and its neurotoxicity has been shown to affect foraging behavior in exposed fish, birds and mammals. Since food limitation is thought to be one of the main causes of larval fish mortality, we investigated the effects of acute 20 hour waterborne methylmercury exposure (0.1 µM, 0.3 µM, 1 µM, 3 µM) on feeding behavior of larval yellow perch. A comparative study was also performed on larval zebrafish (*Danio rerio*). Additionally, we analyzed light/dark swimming activity and swimming speeds of both larval fish species; with these experiments we evaluated the putative role of light/dark perception and overall activity in foraging and prey capture success. Both fish species were studied in order to develop behavioral assays that can transfer easily between an ecologically relevant species and a well-studied fish model. We also looked at the same suite of behaviors of larval yellow perch spawned from fish collected from the wild and measured the corresponding mercury levels. We found that low methylmercury concentrations impaired larval behavior and that this effect could have population implications as suggested when we incorporated these effects into an individual-based model to predict the effects on survival and growth of a cohort. Our results suggest that methylmercury could have an impact on yellow perch recruitment in Lake Michigan.

#### **715 Mercury in Terrestrial Songbirds: species, habitats, and locations at risk**

**A. Jackson**, Biodiversity Research Institute, Oregon State University; D.C. Evers, E. Adams, A. Sauer, Biodiversity Research Institute; C. Eagles-Smith, US Geological Survey / FRES. As research continues to expand on the transfer and fate of methylmercury in ecosystems we now understand that invertivores in terrestrial habitats can be at high risk to atmospheric mercury deposition. By compiling a large dataset of blood mercury levels for songbirds in the northeastern U.S. (N = 3126), we are able to model factors that influence methylmercury availability and apply findings to relatively uncharacterized areas in the central and western U.S. In our northeastern U.S. analysis, we identified five variables that are likely to influence mercury exposure and found, by taking into account species and site differences, that there is a significant effect of habitat type and foraging guild. Fresh- and saltwater wetlands showed significantly higher Hg levels than upland forests and invertivores showed significantly higher Hg levels than omnivores. We can use this information to identify species that would be best used as indicators of greatest risk in different habitat types across the U.S. Such information will better inform land managers, decision makers and governmental regulators concerned about mercury in the environment.

#### **716 Ecological risk of dietary methylmercury exposure to piscivorous fish and wildlife in Canada: A screening level assessment**

**D.C. Depew**, Queen's University / School of Environmental Studies, Queen's University; N.M. Burgess, Environment Canada / Science & Technology Branch, Science & Technology Branch / Environment Canada, Canadian Wildlife Services / Environment Canada; L.M. Campbell, Queen's University / School of Environmental Studies. Deposition of mercury (Hg) from the atmosphere into the environment continues to remain the principle source of Hg contamination for most aquatic ecosystems. Inorganic Hg is readily converted to highly toxic methylmercury (MeHg) that bioaccumulates in aquatic food webs and concentrations in prey fish may be sufficiently high as to pose risks to piscivorous fish and wildlife over large portions of Canada. Using a standardized dietary exposure indicator (Hg<sub>PREY</sub>) modeled from over 200,000 observations of fish Hg concentrations at over 1,900 locations across Canada and recently derived dietary exposure screening benchmarks, we conducted a screening level risk assessment to evaluate the extent and presence of risk to top trophic level piscivores; the common loon (*Gavia immer*), walleye (*Sander vitreus*) and northern pike (*Esox lucius*). Concentrations of Hg<sub>PREY</sub> were sufficiently high enough to exceed benchmark thresholds related to impaired productivity and behaviour in loons at 10 and

36 % of sites respectively, and exceeded benchmark thresholds for impaired reproduction and health in fishes at 82 and 64 % respectively. For both the common loon and piscivorous fishes, the majority of sites deemed to be at risk were located in forested ecozones of eastern Canada known to have been affected by anthropogenic acidification. Results of this assessment suggest that common loons and piscivorous fishes would likely benefit from reductions in Hg emissions.

**717 Mercury Toxicity Thresholds for Birds and Implications for Appropriate Exposure Metrics** P.C. Fuchsman, ENVIRON International Corporation, ENVIRON International Corp.; A.K. Gevertz, ENVIRON International Corp. / Zoology, ENVIRON International Corp. / ESM; A. Fogg, ENVIRON International Corporation; E. Perruchon, ENVIRON International Corp.; M. Cisz, Michigan Technological University; M. Henning, ENVIRON International Corporation. We compiled extensive laboratory and field data linking mercury exposures to effects on birds, with a focus on reproductive effects. The available data have expanded substantially in the last five years. The avian effects data set provides a tool for identifying toxicity reference values for use in risk assessment. In addition, it provides an opportunity to compare the utility of multiple exposure metrics in minimizing interspecies variation, thus improving interspecies extrapolations in risk estimation. Such extrapolation remains a necessity for mercury and is even more frequently required for other, less thoroughly studied chemicals. On the basis of mercury concentrations in eggs, toxicity thresholds are relatively consistent among species, likely because uncertainties of dietary exposure modeling are eliminated. However, egg concentrations are not as frequently available as concentrations in avian dietary items. Therefore we also compared the utility of expressing exposure on the basis of dietary concentrations (mg/kg) versus dietary doses normalized to avian species' body weight (mg/kg BW-day). While body weight scaling of toxicity reference values is no longer widely accepted, body weight normalization of dietary doses remains standard practice. However, the mercury toxicity data support the contention that body weight normalization distorts interspecies comparisons due to extreme differences among species in food ingestion rates relative to body weights.

**718 Mercury in Bats from the Northeastern United States** D. Yates, S. Angelo, D.C. Evers, Biodiversity Research Institute; J. Schmerfeld, U.S. Fish and Wildlife Service, Virginia Field Office; M.S. Moore, T.H. Kunz, Boston University / Center for Ecology and Conservation Biology; T. Divoll, S.T. Edmonds, Biodiversity Research Institute; C. Perkins, University of Connecticut / Center for Environmental Sciences and Engineering; N.O. Driscoll, K.C. Irving Environmental Science Centre; R. Taylor, Texas A&M / Trace Element Research Laboratory. Concentrations of mercury (Hg) and methylmercury (MeHg) in biota from the northeastern United States exist because of atmospheric deposition and anthropogenic point sources. When Hg is methylated it becomes particularly toxic in ecosystem and to the wildlife. This paper examines Hg concentrations found in bat tissues over a four year period in the northeastern United States, and assesses differences sampled near point sources and from nonpoint sources. Percentages of MeHg found in total fur Hg were also evaluated to determine the level of toxicity commonly present in bat tissues. As predicted, Hg was significantly higher in bats captured near point sources compared to nonpoint sources. MeHg made up 86% (range = 71-95%) of the total Hg in 20 bats sampled in northeastern North America where several species are facing threats from white-nose syndrome (WNS), wind-power production, habitat loss, and pollution in air and water, MeHg is a widespread pollutant, and thus it is clear from the present study that a better understanding is needed regarding the neurological and behavioral effects of Hg on bats.

**719 Bioanalytical approach for the characterization of endocrine disrupting compounds in wastewater** S. Dagnino, V. Bellet, M. Grimaldi, V. Cavailles, IRCM, Institut de Recherche en Cancérologie de Montpellier, INSERM, U896; H. Fenet, UMR 5569 Hydrosociences, Université Montpellier 1; P. Balaguer, IRCM, Institut de Recherche en Cancérologie de Montpellier, INSERM, U896. Nuclear receptor based *in vitro* bioassays are useful tools for the detection of endocrine disrupting compounds (EDCs) in environmental samples. Thus, the difficulty lies in the identification of the ligands of such receptors responsible for the measured activity. In the present study, bioanalytical tools were used, in combination with reporter cell lines, in order to characterize EDCs activities in wastewater samples. The occurrence of EDCs such as estrogens in raw urban sewage is usually well

documented, whereas the presence of other receptor ligands has been poorly described. We used ER, PXR, AhR and AR bioassays to characterize EDCs activity in dissolved phase, suspended solids and sludge from a wastewater facility. With bioanalytical characterization, we determined that estrogenic activity was carried out by high affinity compounds in dissolved phase and suspended solids and low affinity compounds in sludge. Sludge revealed to be a complex mixture of ER, AhR, PXR activators and AR inhibitors. Using recombinant ER, and PXR purification column, we were able to determine the contribution of common ER, PXR, AhR and AR ligands and suggested a list of possible compounds responsible for these activities to be considered for further identification.

**720 An Australian-wide assessment of endocrine disruption – stage 1** P.D. Scott, Griffith University / School of Environment; H. Coleman, J. McDonald, University of New South Wales / Water Research Centre; R. Lim, University of Technology Sydney; D. Nuggeoda, RMIT University / School of Applied Sciences, RMIT University / Department of Applied Biology & Biotechnology; L.A. Tremblay, Cawthron Institute; V.J. Pettigrove, The University of Melbourne / CAPIM; H.F. Chapman, Griffith University / School of Environment; S. Blockwell, B. Harper, Sydney Water; T. Reitsema, Department of Water; M. Bartkow, Seqwater; F.D. Leusch, Griffith University / School of Environment. This project uses an integrated approach consisting of multiple *in vitro* and *in vivo* bioassays, *in situ* sampling and trace chemical analysis to compare endocrine disruption at 73 sites in mainland Australia. Sample sites were selected to include waterways impacted by a variety of sources such as wastewater discharge, agricultural run-off, industrial effluent, urban drains and pristine reference sites. Duplicate 1 L discrete water samples were taken quarterly over a one-year period. Samples were concentrated using solid-phase extraction (SPE) and split into two aliquots, one for *in vitro* bioassay analysis and the other for chemical analysis. A battery of CALUX assays (estrogen receptor, ER; androgen receptor, AR; progesterone receptor, PR; and glucocorticoid receptor, GR) was used to determine the classes of EDCs present in water extracts. Chemical analysis was used to identify causative compounds as well as for point source confirmation. Preliminary results have identified at least 11 sites that have estrogenic (or anti-androgenic) EDCs. Analysis with the AR-, PR-, and GR-CALUX assays is currently underway. Chemical analysis has confirmed estrogen mimics (such as bisphenol A, t-octylphenol), the pesticide atrazine, an industrial compound tris(2-carboxyethyl)phosphine (TCEP), and numerous pharmaceuticals and personal care products (e.g., atenolol, dilantin, triclosan, carbamazepine, ibuprofen). Hormone analysis is currently underway. Based on *in vitro* and chemical data, several stock solutions representative of a selection of exposure concentrations found in the environment will be created for *in vivo* laboratory fish exposures using a native species (rainbowfish, *Melanotaenia fluviatilis*) and a widespread exotic pest species (mosquitofish, *Gambusia holbrooki*) in stage 2. An estrogenic biomarker (vitellogenin) and an androgenic biomarker (in development) will be used to assess whole organism endocrine disruption. The same endpoints will be utilized with *in situ* techniques by sampling fish from polluted aquatic environments identified in stage 1 to assess the amount of endocrine disruption present in the most impacted natural aquatic environments. Finally, in stage 3, a risk assessment will be generated using *in vitro*, chemical, *in vivo*, and *in situ* data to assess the potential risk to aquatic ecosystem health.

**721 Evaluation of Endocrine Active Compounds in Wastewater Effluents: implementation of a Mode of Action-based tool-box** R. Marfil-Vega, ORISE Postdoc at US EPA / ORISE Research Participant to the National Risk Management, University of Cincinnati, ORISE Postdoctoral Fellow, ORD, US EPA, ORISE Postdoc at US EPA; M.A. Mills, U.S. EPA, U.S. EPA / National Risk Management Research Laboratory, ORD, US EPA / Remediation and Pollution Control Division, NRMRL, U.S. EPA / Office of Research & Development. Recently researchers have expanded the scope of their work on endocrine disruption to contaminants that may perturb thyroid hormone homeostasis and/or interact with other nuclear steroid receptors (i.e. androgen, progesterone, and glucocorticoid) different from the estrogen one. Concurrently, there has been an increased public awareness and demand of information by citizens and water utilities on the presence of Endocrine Disrupting Chemicals (EDCs) in environmental waters. To properly disseminate the information available and improve the management of the risks associated with the occurrence of EDCs in discharges from wastewater treatment plants (WWTPs), Environmental Sciences (chemistry, and (eco)-toxicology) and Engineering are closely



developing new monitoring strategies. A comprehensive set of tools needs to be implemented in anticipation of Whole Effluent Toxicity requirements of WWTPs to monitor the increasing number of EDCs in wastewater and meet the pertinent regulations. A Mode Of Action-based approach, in which a battery of bioassays and chemical analysis are combined to identify toxic effects and their causing agents, is proposed for this purpose. The current work encompasses a multi-laboratory and interdisciplinary effort to monitor and characterize the endocrine disrupting activity of effluents from WWTPs, and the specific chemicals that may underlie these effects. Outcomes on selected metabolic pathways within the hypothalamus-pituitary-thyroid and hypothalamus-pituitary-gonadal axes are monitored by means of targeted analysis of six groups of chemicals (total 140 target analytes) and a battery of *in vivo*, *ex vivo* and *in vitro* bioassays (12 assays). In this presentation, the occurrence and concentrations of the selected emerging contaminants (steroid and thyroid hormones, perfluorinated chemicals, pharmaceuticals, and alkylphenolic surfactants) during two sampling events, conducted in early spring and summer, in four WWTPs in New England will be reported. Additionally, the comparison between the active samples in the bioassays (i.e. vitellogenin gene expression assay with larvae Fathead Minnows, transgenic amphibian thyroid toxicity assay, thyroid gland explant culture assay, thyroid peroxidase inhibition assay, rat and human pregnane-X, estrogen-, androgen-, progesterone-, glucocorticoid-cell-based nuclear receptor reporter assays, and aromatase inhibition and steroidogenesis assays) and the concentrations of chemicals determined will be discussed.

**722 In Vitro Assessment of Estrogenic Activity in Source and Treated Drinking Water Extracts from 25 U.S Drinking Water Treatment Plants.** V.S. Wilson, US EPA, ORD, NHEERL, TAD / Reproductive Toxicology Branch; N. Evans, US EPA, ORD, NHEERL, Toxicity Assessment Division; K. Schenck, US EPA / NRMRL; H. Mash, U.S. EPA / Office of Research and Development, NRMRL; L. Rosenblum, Shaw Environmental and Infrastructure; S.T. Glassmeyer, U.S. EPA / Office of Research and Development, U.S. EPA / NERL/MCEARD/CERB; E.T. Furlong, U.S. Geological Survey / National Water Quality Laboratory; D. Kolpin, USGS. The presence of estrogenic compounds in environmental water samples and their potential impact on fish, wildlife and human reproductive health has been an ongoing concern. *In vitro* assays have been successfully used to screen for estrogenic activity in many types of water samples including effluents from waste water treatment and cattle, dairy, swine and poultry operations. Few studies, however, have applied *in vitro* assays to evaluate possible estrogenic activity in source and treated drinking water samples. Scientists from the U.S. Environmental Protection Agency (EPA) and U.S. Geological Survey (USGS) are collaborating on a research study to determine the concentrations of contaminants of emerging concern in source and treated drinking waters collected from up to 25 drinking water treatment plants (DWTP) using various treatment processes from across the United States. For this study untreated source and treated drinking water samples from each plant were assessed for cumulative estrogenic activity using T47D-KBluc cells, an estrogen receptor-mediated transcriptional activation (ER-TA) assay. Extracts of each DWTP sample were prepared targeting steroid hormone enrichment. Aliquots of each extract were also retained for analytical chemistry analysis. Spiked (target = 3 pM EEQ in the assay) and unspiked field blank extracts were prepared concurrently with each DWTP extract. Responses for the spiked samples appropriately ranged from 2.8 to 4.7 pM with a mean of 3.6 pM adding confidence in the predictive ability of the assay. Testing of the source and treated water extracts in the ER-TA assay is ongoing. To date low level estrogenic activity has been detected in eight of the source water extracts but post-treatment extracts from the same plant contained either no detectable or significantly reduced estrogenic activity. ER-TA results will be compared to expected estrogenic activity based on analytical chemistry results. Overall this validated, sensitive cell line can be used for screening individual chemicals, mixtures, animal feedlot effluent and drinking water samples. The results allow the prediction of potentially adverse effects in aquatic systems and estimation of potential effects in humans. Disclaimer: This abstract does not necessarily reflect U.S. EPA policy.

**723 Application of an integrated strategy for monitoring of contaminants, including endocrine active chemicals, in Great Lakes Areas of Concern** D.L. Villeneuve, U.S. EPA / National Health and Environmental Effects Research Laboratory, U.S. EPA; G.T. Ankley, US EPA / Office of Research and Development, National Health and Environmental Effects Research Laboratory, Mid-Continent Ecology Division, U.S. EPA / National

Health and Environmental Effects Research Laboratory; J. Berninger, Baylor University / Department of Environmental Science; J. Cavallin, U.S. EPA, ORISE / National Health and Environmental Effects Research Laboratory; E. Durhan, US EPA / Mid-Continent Ecology Division, US-EPA / Research Chemist; K. Jensen, U.S. EPA / National Health and Environmental Effects Research Laboratory; Z. Jorgenson, US Fish and Wildlife Service / Aquatic Toxicology Laboratory, U.S. Fish and Wildlife / Environmental Contaminants; M. Kahl, U.S. EPA / National Health and Environmental Effects Research Laboratory; K. Lee, US Geological Survey; L.M. Thomas, US Fish and Wildlife Service / Mid-Continent Ecology Division. Chemical monitoring strategies are most effective for those chemicals whose hazards are well understood and for which sensitive and cost effective analytical methods are available. Unfortunately, such chemicals represent a minor fraction of those that may currently occur in the environment and impact biological systems. However, in cases such as endocrine disruption, where specific types of biological perturbation have been plausibly and/or empirically-linked to adverse outcomes (i.e., adverse outcome pathways have been characterized), effects-based monitoring approaches can serve as a powerful complement to analytical environmental chemistry for exposure monitoring and diagnostic risk assessments. This presentation describes an integrated research strategy that was employed in five different Great Lakes Areas of Concern (AOCs). The strategy used a combination of four day *in situ* exposures with fathead minnows (*Pimephales promelas*), *in vitro* bioassay-based screening of surface water samples, and analytical quantification of over 150 analytes in surface water and sediment samples to characterize contamination at selected sites within the five AOCs. Biological effects monitoring focused on endpoints associated with reproductive endocrine adverse outcome pathways as well as xenobiotic and steroid metabolism. *In vitro* screening with the MDA-kb2 cell bioassay detected weak androgenic activity at several sites within the lower Fox River and Green Bay AOC. Hepatic cytochrome p4501a1 mRNA induction was observed in fish exposed *in situ* at most sites examined within the St. Louis River, Detroit River, and Maumee River AOCs. In contrast, hepatic vitellogenin mRNA concentrations were rarely elevated in male fathead minnows exposed *in situ*. Ovarian expression of steroidogenesis-related genes was largely un-impacted by *in situ* exposure within the St. Louis River AOC, despite detection of a variety of known endocrine active chemicals and pharmaceuticals in surface waters collected during the period of exposure. On-going research includes the application of unsupervised analyses (e.g., transcriptomics) as well as construction of chemical-gene interaction networks as a means to aid selection of biological endpoints for future effects-based monitoring at these sites. The contents of this abstract neither constitute nor reflect official US EPA policy.

**724 A methodological approach to weight of evidence in ecotoxicological risk assessment** K. Solomon, University of Guelph / School of Environmental Sciences, University of Guelph / Centre for Toxicology, School of Environmental Sciences; M. Hanson, University of Manitoba / Department of Environment and Geography, University of Manitoba / Faculty of Environment; A. Hosmer, Syngenta Crop Protection, LLC., Syngenta Crop Protection, Inc.; W. Kloas, IGB; G. Van Der Kraak, University of Guelph / Department of Integrative Biology. The term "weight of evidence" (WoE) is widely used in human and ecological risk assessment but is seldom applied properly. Most of the literature uses the term metaphorically. WoE is not a simple vote-counting exercise but is an interpretative methodology for systematically assessing studies using quality pre-determined criteria for: 1) the relevance of a response to a specific endpoint and, 2) the strength of the methods used in the study from which the data were derived. We developed a process for WoE that encompasses all available evidence, rather than some arbitrarily selected subset, and assesses this in a quantitative way. We developed numerical criteria to characterize adverse outcomes(s) in terms of biological relevance of the response and strength of methods used to measure the response. Relevance was characterized quantitatively using criteria based on statistical significance, response to concentration, consequences for survival, growth, development, and reproduction, and mechanistic understanding of mode of action. Strength of the study was then characterized using procedures recommended for assessing studies for inclusion in databases such as IUCLID, the use of GLP and QA/QC, numbers of concentrations used, and the inclusion of realistic and upper-bound values in the range of concentrations tested. The means of the scores of these two assessments were then used to summarize and weigh the evidence in terms of adverse outcomes and the strength of the methods used to derive these data. To derive a weight of evidence that was

easily interpreted visually, these scores were plotted on the X- and Y-axes of a graph. The locations of the plotting points allow all of the studies that reported a particular response to be visually displayed for summary evaluation. Clustering of the studies in a particular part of the graphic is indicative of consistency between observations across studies. Obvious outliers or exceptions can easily be identified and, if necessary, addressed in a narrative. This process will be illustrated using atrazine as a case compound.

**725 Use of an In Vitro Nuclear Receptor Assay Panel to Characterize the Endocrine-Disrupting Activity Load of Wastewater Treatment Plant Effluent Extracts** K.B. Paul, The Hamner Institutes for Health Sciences / Institute for Chemical Safety Sciences, Integrated Systems Toxicology Division / NHEERL, ORD, US EPA, ORISE Postdoctoral Fellow, ORD, US EPA / Integrated Systems Toxicology Division, NHEERL; R. Marfil-Vega, ORISE Postdoc at US EPA / ORISE Research Participant to the National Risk Management, University of Cincinnati, ORISE Postdoctoral Fellow, ORD, US EPA, ORISE Postdoc at US EPA; M.A. Mills, U.S. EPA, U.S. EPA / National Risk Management Research Laboratory, ORD, US EPA / Remediation and Pollution Control Division, NRMRL, U.S. EPA / Office of Research & Development; S.O. Simmons, ORD, U.S. EPA / Integrated Systems Toxicology Division, NHEERL; V.S. Wilson, US EPA, ORD, NHEERL, TAD / Reproductive Toxicology Branch, U.S. EPA / Office of Research and Development, ORD, US EPA / Toxicity Assessment Division, NHEERL, U.S. EPA, ORD, NHEERL / Toxicity Assessment Division; K.M. Crofton, ORD, US EPA / Integrated Systems Toxicology Division, NHEERL. The objective of this multi-laboratory project is to characterize the potential endocrine-disrupting activity of wastewater treatment plant (WWTP) effluent extracts from the Assabet River (Boston, MA) using bioassays and analytical chemistry. We tested the hypothesis that estrogen receptor (ER), androgen receptor (AR), and pregnane-X receptor (PXR) assays would provide a sensitive screen for the potential estrogenic, androgenic, and thyroid-disrupting load of samples obtained from WWTP effluents and aid prioritization of further analytical and biological testing of these effluent extracts. Effluent samples from 4 WWTP sampling sites and a blank were extracted using 6 different protocols, each targeting specific chemical groupings. In order of descending potency, extracts enriched for thyroid hormones, pharmaceuticals, aromatase inhibitors, steroids, and alkylphenols tested positive for estrogenic activity (4-10 ng/L estradiol equivalents, EEQs), and with greater efficacy than the perfluorinated chemicals extracts (> 1 ng/L EEQ). The potency of ER activation by each extract type was WWTP site-dependent. Several extracts demonstrated modest AR agonist activity, generally coincident with extracts from the site that demonstrated the highest ER activation. Significant AR antagonism was not observed for any extract. Preliminary data obtained with a PXR activity assay suggests that the pharmaceutical extract method may be most appropriate for subsequent assays of potential thyroid-disruptors that up-regulate hepatic catabolism of thyroid hormones. This was consistent with previous work that demonstrated PXR activation 3-fold over vehicle control with whole freeze-dried effluents at environmental concentrations. Continuing work will relate activation of ER, AR, and PXR to identify possible potency relationships between these activities in the effluent extracts. Extracts will also be tested in additional bioassays, including a thyroid peroxidase inhibition assay, to assess another thyroid disruption target using an emerging medium-throughput technology. These data inform an integrated bioassay and analytical approach to understanding the potential endocrine-disrupting activity of effluents and potential strategies for monitoring EDC activities following changes in treatment technologies. This abstract does not necessarily reflect the policy of the US EPA.

**726 How reliable monitoring tool can be the estrogenic in vitro assay** ? B. Jarosova, Masaryk University, Research Centre for Toxic Compounds in the Environment / RECETOX, Masaryk University / Faculty of Science, RECETOX; K. Hilscherova, Masaryk University, Research Centre for Toxic Compounds in the Environment / RECETOX; R. Loos, B. Gawlik, EC JRC Ispra, Italy; J.P. Giesy, University of Saskatchewan / Biomedical Veterinary Sciences & Toxicology Centre, Michigan State University / Department of Animal Science, University of Saskatchewan / Toxicology Centre; L. Blaha, Masaryk University, RECETOX / RECETOX, Masaryk University, Research Centre for Toxic Compounds in the Environment / RECETOX, Masaryk University / RECETOX. Although the presence of estrogenic compounds in the environment has been extensively studied in the last decades, many questions related to their risk have not been fully

resolved yet. E.g. instrumental analyses of steroidal estrogens, which appear to be primarily responsible for at least some adverse effects observed in fish downstream of Waste Water Treatment Plants (WWTPs), often fail in detection of their actual concentrations in complex environmental matrices and/or their costs are burdening. In 2010, FATE-SEES pan-European monitoring campaign coordinated by the European Commission's Joint Research Centre (JRC) in Ispra, Italy, was conducted. Samples from 90 European Waste Water Treatment Plant (WWTP) effluents were collected and analyzed for more than 150 polar organic and 20 inorganic compounds to obtain an overview on many so far only locally investigated compound classes. Steroidal estrogens were not detected in any of these samples above the detection limit of 10 ng/L. However, the effect-based monitoring approach (MVLN *in vitro* assay) showed significant estrogenic activity of 27 out of 75 samples with E2 equivalent ranging 0.53 to 17.9 ng/L. The greatest activities have been detected in WWTPs at some of the major European cities indicating the importance of this contamination source. The results were further combined with upgraded literature review of typical concentrations of individual major estrogens in European municipal waste water effluents and their *in vitro* and *in vivo* relative potency to E2 to evaluate usefulness of the *in vitro* assay for environmental monitoring and to answer the question; how they can be interpreted in relation to potential *in vivo* adverse affects.

**727 Area use, site fidelity, and rate of movement of white croaker (*Genyonemus lineatus*) within the Los Angeles and Long Beach Harbor** B. Ahr, M. Farris, C.G. Lowe, California State University Long Beach / Department of Biological Sciences. The Los Angeles (LA) and Long Beach (LB) Harbor in southern California is known to be an area of contaminant accumulation, both from industrial/agricultural and residential sources. This study employs acoustic telemetry techniques to gain insight into the short-term and long-term movements of white croaker (*Genyonemus lineatus*), a well known indicator species. To characterize longer-term, more course-scale movements, the harbors were divided into four areas; inner LA, outer LA, inner LB, and outer LB, and 15 individuals were tagged in each section. Their movements between sections was monitored using 12 acoustic receivers spread throughout the harbors. Short-term, fine-scale movements are studied using active acoustic tracking, where fish are followed over multiple 24 hr periods. Individuals actively tracked use an avg. daily area of 90,600 m<sup>2</sup> ± 57,740 m<sup>2</sup> (± SD). So far, no significant difference has been observed in area use during day time (50,887 m<sup>2</sup> ± 38,315 m<sup>2</sup>) and night time (48,340 m<sup>2</sup> ± 38,394 m<sup>2</sup>) (p = 0.798). Longer-term, course-scale monitoring has indicated that *G. lineatus* tagged in the inner LA area exhibited highest site fidelity, remaining in the area in which they were tagged for 29.46% ± 11.31% (± SD) of the days at liberty during the study period. The lowest site fidelity observed so far was in the outer LB area, where tagged fish remained present for 10.82% ± 11.88% of the days at liberty during the study. Differences in site fidelity were found to be highly significant (p = 0.00038). The average number of detections per fish, was highest (16,518) at Station 6, located in an area of the inner LA Harbor known as the Consolidated Slip. This receiver station has also logged the highest number of total detections from all fish at 611,154 so far. These preliminary results point to the Consolidated Slip as a highly utilized area by *G. lineatus* in the Harbor. This area is also known to contain the highest concentrations of DDT and PCBs in sediments throughout the entire Harbor.

**728 Dominguez Channel and Greater Los Angeles and Long Beach Harbor Waters Toxic Pollutants TMDL, a Summary of the Regulatory and Environmental Overview** M. Arms, Port of Long Beach / Environmental Management Division; A. Jirik, K. Curtis, Port of Los Angeles.

Total Maximum Daily Loads (TMDLs) are regulatory tools that have been successfully applied in many instances across the nation with the end result of a cleaner environment. TMDLs were developed to address point source contamination, but are challenging to implement for non-point source or legacy contamination situations. TMDL development in the Los Angeles area has been driven by a consent decree signed between the United States Environmental Protection Agency (USEPA), Heal the Bay, Inc. and Santa Monica BayKeeper, Inc. in 1999 that required all 1998 Clean Water Act Section 303(d) listed waterbodies to have TMDLs completed within 13 years of the consent decree. The TMDL for Toxic Pollutants in Dominguez Channel and Greater Los Angeles and Long Beach Harbor Waters is one of the 92 TMDLs under the consent decree. This TMDL is complicated by the number of environmental matrices (water, sediment, and tissue), number of stakeholders, presence of legacy pollutants, questions regarding



the bioaccumulation and human health risk of the pollutants, and questions regarding any current inputs of the TMDL pollutants into the system. Because of the complexities of the TMDL, many questions were not completely answered prior to adoption of the TMDL. The purpose of this talk is to summarize the final TMDL, effective March 2012 (with a 20-year implementation), as well as outline the remaining questions and the proposed scientific studies that need to be conducted in order to better inform the TMDL.

**729 Estimating Fish Tissue Concentrations from Sediment-Associated Contaminants: Incorporating Fish Behavior** K. von Stackelberg, E Risk Sciences, LLP. It is important to understand the spatial distribution of non-point sources to sediments and how that influences exposure and potential trophic transfer of contaminants. Aquatic food web exposures to sediment-associated contaminants depend on contaminant concentrations but also the foraging strategies of the fish species. These foraging and migration strategies can lead to differential exposures that are not captured through the use of simple exposure statistics such as means or SWACs. This presentation describes the use of spatially explicit modeling approaches to simulate the effect of fish foraging strategies and habitat use on predicted tissue concentrations. A range of scenarios are explored to highlight the quantitative influence of including fish behavior and capturing spatially-explicit exposures when considering TMDLs.

**730 Modeling Bioaccumulation of Contaminants in Puget Sound: An Analysis of Site-Specific Parameters** G. Lowery, Western Washington University / Huxley College of the Environment, Western Washington University; E. Patmont, Western Washington University / Huxley College of the Environment; R.M. Sofield, Western Washington University / Huxley College of Environment. The industrial activity in the Puget Sound region necessitates understanding the bioaccumulation of contaminants to achieve protective levels for aquatic life and safe fish consumption. Considerable progress has been made in modeling the bioaccumulation of contaminants in aquatic organisms; because of this, there are improved theory and methods to assess the contributing parameters and identify data gaps. In the current study, the extensive data for Puget Sound biological and environmental characteristics were compiled from the Washington State Department of Ecology's Environmental Information Management System (myEIM) for input parameters. The AQUAWEB model of Gobas and Arnot was built in STELLA (ISEE Systems, Inc., Lebanon, New Hampshire; www.iseesystems.com). As a preliminary modeling step, the distribution of each input parameter was determined using Oracle Crystal Ball. The median of each parameter was used in the STELLA® model. STELLA was also used to run a sensitivity analysis. The results are a predicted sum PCB concentration of 155 ng chemical/g of organism in *Mytilus edulis* for three common PCB mixtures of Aroclor 1242, 1254, and 1260. The model was substantially more sensitive to percent sediment organic carbon (a site-specific value) while organism parameters such as mussel weight and lipid content, and other site-specific environmental parameters including water temperature, particulate organic matter, dissolved organic matter, and the concentration of suspended solids had virtually no impact on the model output. To further characterize what concentrations of PCBs are likely to bioaccumulate in *M. edulis* from Puget Sound, a Monte Carlo analysis will be completed using the parameter distributions. Model calibration will be included using measured bioaccumulation in a subset of the Puget Sound dataset.

**731 Overview of Monitoring Conducted in Support of the Los Angeles and Long Beach Harbors Toxics TMDL** A. Jirik, K. Curtis, Port of Los Angeles; M. Arms, Port of Long Beach; A. Crumpacker, D. Moore, Weston Solutions, Inc. The recently adopted Los Angeles and Long Beach Harbors Toxic Total Maximum Daily Load (TMDL) requires that special studies, including harbor-specific sediment and fish tissue linkage studies, will be developed and implemented within the Harbor Complex. These studies are crucial to determine allowable sediment contamination levels in the Harbor Complex which will result in compliance with fish tissue numeric targets. Compliance with the TMDL will likely be based on sediment quality objectives (SQOs), which were adopted by the California State Water Resources Control Board in 2009 to assess the direct effects of pollution in sediments on benthic communities. The direct effect SQOs use a multiple lines of evidence approach and include sediment chemistry, sediment toxicity, and benthic community assessments. Currently, Phase 2 of the SQOs is under

development, and focuses on the indirect effects of sediment contamination. Critical to TMDL implementation is that the SQO Phase 2 decision support tool focuses on prediction of the proportion of fish tissue contamination from sediment pollution. Within the Harbor Complex, it is not currently known how much of the observed fish tissue contamination can be linked to local contaminated sediments. This makes compliance with the TMDL, which requires clean-up of contaminated sediments to levels which will result in fish tissue numeric target compliance, difficult. The Ports of Los Angeles and Long Beach have devoted significant resources to the development of a hydrodynamic, water quality and sediment transport model, fish tracking studies, and sediment collection, analysis and reporting in support of the TMDL. In 2011, fish tissue, sediment, and overlying water samples were collected for the purpose of developing a sediment and fish tissue linkage analysis. This dataset represents the first major effort in the State focused on collecting data for the purpose of developing sediment and fish tissue linkage. This presentation focuses on initial linkage evaluations between sediments and observed fish tissue concentrations as well as a comparison of the 2011 results to Palos Verdes shelf studies. Final results of these analyses will be important for the development of the TMDL monitoring and implementation plans, as well as to calculate revised sediment numeric targets.

**732 Sediment Transport: The Key to Understanding Contaminants in Sediments for TMDL Applications** Y. Poon, S. Ueoka, Everest International Consultants; A. Jirik, Port of Los Angeles; K. Curtis, Port of Los Angeles / Environmental Management Division; M. Arms, Port of Long Beach / Environmental Management Division. The Dominguez Channel and Greater Los Angeles and Long Beach Harbor Waters (Harbor) Toxic Pollutants Total Maximum Daily Loads (TMDLs) will limit the discharge of pollutants into San Pedro Bay with the goal of meeting water quality standards. Impairments are attributed to heavy metal and organic pollutant concentrations in existing sediments and organic pollutant levels in fish tissue. Fundamentally, the Harbor TMDLs were developed based on the concept that pollutant concentrations in the sediment bed are the result of depositing sediment and contaminants, and there is a direct linkage between the contaminants in the sediment bed to the fish tissue. Hence, an understanding of the sediment loadings to the harbor from watersheds and storm water runoffs, as well as the erosion and migration of the resuspended sediment from existing harbor bed, is important for the interpretation and implementation of the Harbor Toxic TMDL. This paper provides a summary of recent studies conducted by the Port of Los Angeles and the Port of Long Beach (Ports) and the others to understand the loading, erosion and transport of sediments in the Harbor. These studies include field works and the development of a three-dimension (3-D) hydrodynamic, sediment and contaminant transport for the Harbor. The field studies include: (i) an ongoing sediment tracer study to assess the erosion of the sediment bed in the Dominguez Channel and Consolidated Slip; the study aims at understanding erosion of bed sediments and the transport of contaminants associated with the eroded bed sediments into the Harbor using synthetic tracers to mimic the sediment properties and tracking the erosion and migration of the tracers into the Harbor under both dry and wet weather conditions; (ii) a sediment tracer study to assess deposition of sediments in the Harbor from the Los Angeles River and San Gabriel River during rain events; and (iii) a study to understand the dispersion of sediment plumes due to dredging activities in the Harbor. The data collected from these field studies were also used to calibrate the 3-D hydrodynamic and sediment transport model.

**733 Tracking, Identifying, and Abating Non Point Source Pollution, the Big Question for TMDL Compliance** G. Williams, D. Renfrew, Weston Solutions, Inc. Agencies and municipalities facing Total Maximum Daily Load (TMDL) implementation and compliance are required to develop implementation plans that identify sources and provide solutions to abate point and non-point pollutant sources. Requirements to abate or reduce sources are set on an implementation timeline as part of the TMDL and require coordination among stakeholders. Non-point sources of pollution are some of the most difficult to control, because of the complexities required to identify, quantify, and abate them. In the case of air deposition and stormwater runoff, multiple jurisdictions, regulatory agencies, and potential pollutant sources must all be considered during TMDL implementation planning. The load allocations for air deposition of pollutants included in the TMDL for Toxic Pollutants in Dominguez Channel and Greater Los Angeles and Long Beach Harbor Waters (Toxics TMDL) is greater than the



load allocation from sediments for some pollutants included in the TMDL. No reductions of air deposition are required within the TMDL. A similar situation can be found for the Chollas Creek Dissolved Metals TMDL, which is located in the City of San Diego. To address the uncertainty of air deposition, and its importance as a non-point source contributing to elevated dissolved copper levels in the Creek, the City of San Diego conducted a multiple phased aerial deposition and pollutant source identification study to better understand surface water pollutant source contributions in the Chollas Creek Watershed. Findings from the study demonstrated numerous air deposition sources from industries, transportation, facilities and architectural building materials. These aerial sources can be collectively considered non-point sources even though they originated from a given location and were transported through an alternative transport mechanism (air deposition versus stormwater runoff). The study showed that 49% of the copper load found in urban runoff from Chollas Creek was attributable to automobile brake pad wear from the overall watershed while 51% of the copper load was attributable to specific *permitted* industrial source emissions. Because atmospheric deposition can be a significant contribution to TMDL loads, both directly and indirectly through stormwater runoff, mechanisms to quantify, identify sources, and abate these sources are necessary for successful TMDL implementation and ultimately, compliance.

**734 Root pore size as a potential mechanism of exclusion for gold nanoparticle uptake in aquatic vascular plants** B. Glenn, Clemson University / Clemson Institute of Environmental Toxicology (CU-Entox), Clemson Institute of Environmental Toxicology / graduate student; J. Hudson, Advanced Materials Research Laboratories (AMRL); S.J. Klaine, Clemson University / Institute of Environmental Toxicology (CU-ENTOX), Clemson Institute of Environmental Toxicology / Institute of Environmental Toxicology (ENTOX), Clemson University / Institute of Environmental Toxicology (ENTOX). The three aquatic plants *Egeria densa*, *Myriophyllum simulans*, and *Azolla caroliniana* have been found to bioconcentrate spherical, citrate capped gold nanoparticles (AuNPs) in suspension. The bioconcentration factor of AuNPs was found to be dependent on size of AuNP and species of aquatic plant. The AuNP treatments used in this study had an average size of 4, 18 and 30 nanometers (nm). To determine a possible mechanism of exclusion, the roots of each species were further investigated. The root structure was investigated using a published solute exclusion technique to estimate root pore size. Based off of size of solute, and principles of diffusion, the change in root cell shape can be linked to the cellular wall pore size. This estimation of pore size was then confirmed using high-resolution transmission electron microscopy (TEM) to visualize the pore structure in the roots of each species. Based on previous research, exposure to AuNPs in well water at a nominal concentration of 250 µg/L showed highest bioconcentration of small AuNPs and the least bioconcentration of the large AuNPs in each species. *Azolla caroliniana* showed the highest tissue concentrations of the species tested. Results indicated that pore size plays an important role in AuNP uptake, excluding the larger particles. While root pore size was important in initial AuNP uptake, results also indicated that plant physiology played an important role in concentration of AuNPs into the plant tissue. Aquatic plants with a transpiration stream tended to show higher bioconcentration factors than those that were fully submerged.

**735 Impact of Cerium Oxide Nanoparticles on Cilantro (*Coriandrum sativum*)** M.I. Morales, A.C. Barrios, C.M. Rico, J.R. Peralta-Videa, J.L. Gardea-Torresdey, The University of Texas at El Paso / Department of Chemistry. The impact of engineered nanoparticles (ENPs) on edible plants is currently a theme of great concern. Studies have reported that CeO<sub>2</sub> NPs are stored in plant tissues without modification. Thus, these NPs could be distributed in the food chain through edible plants, posing a threat for human health. Cilantro (*Coriandrum sativum*) is a very important culinary and medicinal plant worldwide. It is consumed either as a fresh herb or as a spice. In this research cilantro plants were germinated and grown in organic soil treated with CeO<sub>2</sub> NPs at concentrations varying from 0 to 500 mg kg<sup>-1</sup>. Spectroscopic techniques were used to analyze the plants treated with CeO<sub>2</sub> NPs. The data showed a significant difference in the uptake of the CeO<sub>2</sub> NPs at 250 and 500 mg kg<sup>-1</sup> compared to 62.5 and 125 mg kg<sup>-1</sup>. In addition, plants treated with 125 and 250 mg Kg<sup>-1</sup> of CeO<sub>2</sub> NPs were significantly longer compared with controls and plants treated with 62.5 and 500 mg kg<sup>-1</sup> CeO<sub>2</sub> NPs.

**736 Impact of functionalized and non-functionalized multiwalled carbon nanotubes on arsenic hyperaccumulation in Chinese brake fern (*Pteris vittata*)** B. Shrestha, Texas Tech University / Department of Environmental Toxicology, Texas Tech University; P. Payton, USDA-ARS; J. Canas, Texas Tech University / Department of Environmental Toxicol. Arsenic (As) is a widely distributed and highly toxic contaminant in the environment. Carbon nanotubes (CNTs) are considered a good adsorbent for inorganic and organic ions. Surface modified, raw and functionalized CNTs have been found to adsorb anionic heavy metals like As and chromium. Hence, the presence of CNTs in soil may modify the bioavailability of anionic As, which may ultimately affect As hyperaccumulation in plants, including food crops, from soil. Considering the potential release of CNTs into the environment, it is important to understand their effect on the fate of contaminants, like As. In addition, evaluation of potential application of CNTs in green technology, such as phytoextraction of cationic and anionic heavy metals from soil and groundwater, is innovative for environmental remediation. This study aimed to evaluate the effects of functionalized and non-functionalized multiwalled carbon nanotubes (MWNT) on As hyperaccumulation in Chinese brake fern (*Pteris vittata*, an As hyperaccumulator). Clean field soil was spiked with potassium arsenate monobasic and aged for four weeks at 60% water holding capacity. Soil samples were taken to determine initial total As concentrations. Arsenic-spiked soil was spiked with functionalized and non-functionalized MWNT at four concentrations (0-, 25-, 50-, 100 mg/kg). Chinese brake fern (four to five frond stage) was planted in a pot containing the As-MWNT mixture and grown for 4 weeks in a greenhouse at 16:8 hours light:dark at 60% water holding capacity. Fern were harvested after 4 weeks and dried at 65°C for 48 h. Fern roots and fronds were digested and analyzed using EPA Method 3050B. Translocation and bioaccumulation factors for As in the fern was calculated based on the ratio of total As concentration in fronds to roots and plants to soils, respectively. This research will provide valuable information on the effects of MWNT on As bioavailability in soil as well as on the potential application of MWNT in green technology for environmental remediation.

**737 Hg bioaccumulation in shoots of the macrophyte *Elodea nuttallii*: an opportunity for phytoremediation?** N. Regier, University of Geneva; C. Cosio, Geneva University, Geneva University / Forel Institute. Previous studies suggest that macrophytes might participate in bioaccumulation and biomagnification of toxic mercury (Hg) in aquatic environment. Hg bioaccumulation and uptake mechanisms in macrophytes need therefore to be studied. Amongst several macrophytes collected in an Hg contaminated reservoir in Romania, *Elodea nuttallii* showed a high organic and inorganic Hg accumulation and was then further studied in the laboratory. *Elodea nuttallii* is a rooted submerged aquatic macrophyte native to North America, which occurs as aggressive invader worldwide. It is often found in metal contaminated sites. Hg tolerance and accumulation of this plant was also high in the microcosm. Basipetal transport of inorganic Hg was predominant, whereas acropetal transport of methyl-Hg was observed with apparently negligible methylation or demethylation *in planta*. Hg concentrations were higher in roots>leaves>stems and in top>middle>bottom of shoots. In shoots, more than 60% Hg was found intracellularly where it is believed to be highly available to predators. Accumulation of inorganic and methyl-Hg in shoots was highly reduced by cold, death and by competition with Cu<sup>2+</sup> for inorganic Hg. Hg in *E. nuttallii* shoots seems to mainly originate from the water column, but methyl-Hg can also be remobilized from the sediments and might drive in part its entry in the food web. At the cellular level, uptake of Hg into the cell sap of shoots was linked to the metabolism, in particular to copper transporters. In summary, our results revealed that shoots of *E. nuttallii* are extremely tolerant to Hg and show huge bioaccumulation ability. Both might be interesting for phytoremediation of contaminated water. Based on densities previously reported in the field by other authors (350-2800 g DW m<sup>-3</sup>) and concentration of Hg found in Babeni reservoir, it can be estimated that 0.7 to 5.6 mg Hg m<sup>-3</sup> could be removed with these plants. This amount is likely to return to water and sediments at the end of the growing season when plants decay. Moreover, the Hg uptake by shoots might be an important pathway of Hg from the water to organisms feeding on the macrophytes. In conclusion, these plants should probably be removed in contaminated sites.

**738 Plant uptake, translocation and bioaccumulation of PPCPs in vegetables** X. Wu, J.L. Conkle, J. Gan, University of California, Riverside / Dept. of Environmental Sciences. In arid and semi-arid regions (e.g.,

American Southwest), municipal treated wastewater is becoming an important water source to augment agricultural irrigation. An emerging threshold issue to this reuse, however, is the potential accumulation of trace contaminants such as pharmaceutical and personal care products (PPCPs) from the recycled water to edible plant tissues. At present very few studies have considered the uptake of PPCPs by plants, and our knowledge of plant accumulation, especially in vegetables, is essentially nonexistent. In this study, we evaluated the uptake and translocation of 19 PPCPs which were frequently detected in treated wastewater by four common vegetables (iceberg lettuce, spinach, cucumber and pepper) that were grown in spiked nutrient solutions under greenhouse conditions. The spiked concentrations were 500 ng/L and 5 µg/L, with the lower treatment level close to those in real treated wastewater. The result showed that many PPCPs could be accumulated by vegetables via root uptake. Fluoxetine accumulated in both leaves and roots, while triclocarban and triclosan tended to accumulate in roots. Some PPCPs like caffeine, meprobamate, diuron, carbamazepine, were more likely to accumulate in leaves. The accumulation potential and the translocation tendency from roots to leaves were further evaluated by correlating with the properties of PPCPs, e.g., log  $K_{ow}$ .

**739 Uptake of pharmaceutical and personal care products by cabbage (*Brassica campestris*) from fortified and biosolids-amended soils** C. Holling, Colorado State University-Pueblo / Biology; J.L. Bailey, Colorado State University-Pueblo / Chemistry; B. Vanden Heuvel, Colorado State University-Pueblo / Biology; C.A. Kinney, Eastern Washington University / Chemistry & Biochemistry, Colorado State University-Pueblo / Chemistry. Pharmaceuticals and personal care products (PPCPs) are commonly present in wastewater biosolids and subsequently present in soil amended with biosolids. To measure the potential for PPCPs to accumulate in crops, two sets of greenhouse experiments to study the accumulation of PPCPs in soils or biosolids by Chinese cabbage (*Brassica campestris*) were. In the first set of experiments the soil was fortified with four pharmaceuticals to environmentally relevant concentrations based on typical biosolids application rates. This resulted in final soil concentrations of 2.6 ng g<sup>-1</sup> carbamazepine, 3.1 ng g<sup>-1</sup> sulfamethoxazole, 5.4 ng g<sup>-1</sup> salbutamol, and 0.5 ng g<sup>-1</sup> trimethoprim. In the second set of experiments cabbage was grown in soil amended with an agronomic rate of biosolids from a local wastewater treatment plant. The concentration of PPCPs in the biosolids-amended soil was 93.1 ng g<sup>-1</sup> carbamazepine, 67.4 ng g<sup>-1</sup> sulfamethoxazole, 30.3 ng g<sup>-1</sup> salbutamol, 433.7 ng g<sup>-1</sup> triclosan and 24.7 ng g<sup>-1</sup> trimethoprim. After growing to maturity the plants in each study were harvested and the arials were separated from roots for individual analysis. All four of the human pharmaceuticals in the fortified soil were detected in both the aerial and root tissues of the cabbage. The median concentrations of pharmaceuticals in the cabbage tissues were: 255.4 ng g<sup>-1</sup> arials and 272.9 ng g<sup>-1</sup> roots carbamazepine; 110.8 ng g<sup>-1</sup> arials and 226.7 ng g<sup>-1</sup> roots sulfamethoxazole; 47.6 ng g<sup>-1</sup> arials and 91.6 ng g<sup>-1</sup> roots salbutamol; and 7.6 ng g<sup>-1</sup> arials and 53.7 ng g<sup>-1</sup> roots trimethoprim. Although all study compounds were present in the biosolids-amended planting soil, only carbamazepine (317.6 ng g<sup>-1</sup> arials and 416.2 ng g<sup>-1</sup>), salbutamol (21.2 ng g<sup>-1</sup> arials and 187.6 ng g<sup>-1</sup>), and triclosan (22.9 ng g<sup>-1</sup> arials and 1220.1 ng g<sup>-1</sup> roots) were detected in the arials and roots of the cabbage. Sulfamethoxazole was also detected in the roots of one of the plants grown in the biosolid-amended soil. The results of this study demonstrate that plants exposed to environmentally relevant concentrations of the PPCPs can accumulate these compounds including in the edible portion of the plant.

**740 Evaluation of Extraction Methods on the Characterization of Antibiotic Uptake in Lettuce** J.B. Sallach, University of Nebraska-Lincoln / Department of Civil Engineering; S. Bartelt-Hunt, University of Nebraska / Civil Engineering, University of Nebraska-Lincoln / Civil Engineering; D. Snow, University of Nebraska-Lincoln / Water Sciences Laboratory, School of Natural Resources, University of Nebraska / Associate Professor; X. Li, University of Nebraska-Lincoln / Civil Engineering; L. Hodges, University of Nebraska-Lincoln / Agronomy and Horticulture. Global water scarcity necessitates the reuse of municipal or agricultural wastewater for irrigation. Typically, municipal and agricultural wastewater can contain low levels of antibiotics and these compounds can be introduced to plants after irrigation with recycled wastewater. In addition, there is little information on the optimal methods for extraction of antibiotics from complex cellulosic matrices, such as plant tissues. In this study, the potential uptake of four antibiotics originating in irrigation water was evaluated. The recovery of four antibiotics (sulfamethoxazole, oxytetracycline, lincomycin, and ciprofloxacin)

was optimized by comparing four extraction techniques: freeze and thaw, mechanical maceration, ultrasonication, and microwave assisted solvent extraction (MASE). To evaluate the extraction techniques, lettuce samples were spiked with a known quantity of each antibiotic and corresponding internal standards. Extracts were analyzed by electrospray ionization LC-MS/MS and method detection limits (MDL) and percent recoveries were determined. Initial results show MASE having the least efficient extraction, perhaps resulting from increased temperatures. Ultrasonication resulted in the lowest MDLs and highest percent recovery for the target antibiotics, followed by the freeze and thaw method. After development of the extraction approach, the presence of antibiotics in lettuce grown in a potting soil was monitored after subirrigation for 6 weeks with a simulated wastewater containing 1 mg/L of ciprofloxacin, lincomycin, oxytetracycline, and sulfamethoxazole. Initial results indicate that antibiotics from recycled wastewater can be transferred to plant tissue. The relative quantities of antibiotics in lettuce shoots and rhizosomal soil will be reported. Preliminary results indicate potential antibiotic uptake on the order of 10<sup>-10</sup> ng/g of plant material.

**741 Investigating differences in the root to shoot transfer of organic compounds between zucchini, squash and soybean using a pressure chamber method** W.J. Doucette, N. Orito, Utah State University / Utah Water Research Laboratory. Transpiration Stream Concentration Factors (TSCF) for 14C-caffeine (log K<sub>ow</sub> = -0.07), 14C-triclocarban (log K<sub>ow</sub> = 4.9) and 14C-endosulfan (log K<sub>ow</sub> = 3.83), relative to titrated water, were determined using a pressure chamber method for soybean (*glycine max L.*), zucchini (*cucurbita pepo ssp pepo*), and squash (*cucurbita pepo ssp ovifera*). TSCF values for caffeine (TSCF = 0.8), endosulfan (TSCF = 0.2) and triclocarban (TSCF = 0.05) were statistically equivalent for squash and soybean. For zucchini, the TSCF for caffeine was the same as for squash and soybean. However, for the more hydrophobic endosulfan and triclocarban, the TSCF values were 0.6 and 0.4, or about 3 and 10 times greater, respectively, than the soybean and squash. The greater difference in TSCF with increasing log K<sub>ow</sub> suggested a solubility enhancement in the zucchini xylem sap. To test this hypothesis, the solubility of caffeine and triclocarban in soybean and zucchini xylem sap was determined and compared to deionized water using a modified shake flask method. Caffeine solubility in the xylem saps of soybean and zucchini was equal to deionized water (22000 mg/L) triclocarban solubility in the zucchini xylem sap was double (20.6 mg/L) that of the soybean xylem sap (10.6 mg/L) and deionized water (11.2 mg/L). This indicates that the enhanced root to shoot transfer of hydrophobic organics reported for zucchini may be partly due to increased solubility in the xylem sap. Further xylem sap characterization is needed to determine the mechanism of solubility enhancement.

**MP001 A comparison of food web magnification factors for mercury transfer through communities within two estuaries of Southwest Florida** D. Rumbold, Florida Gulf Coast University / Associate Prof of Marine Science, Florida Gulf Coast University / Professor of Marine Science; T. Lange, D. Richards, Florida Fish and Wildlife Conservation Commission; H. Benedict, Florida Gulf Coast University. We are assessing mercury (Hg) trophic transfer within different ecosystems of southwest Florida using stable isotope analysis (SIA) and the food web magnification factor (FWMF; also known as trophic magnification factor) approach. Viewed as an average biomagnification factor through a community, FWMFs for Hg can differ between systems as a result of variation in food web dynamics (e.g., length, linkage strength, etc.) or methylation potential and basal concentration of Hg entering the food web. Here we compare  $\delta^{15}\text{N}$ -derived slopes and FWMF for the Shark River and Caloosahatchee River estuaries that have watersheds with very different land uses. Although the hydrology of both has been altered, the former has relatively pristine habitats and comprises the major water flow pathway through southern Everglades National Park (ENP), whereas the later watershed is dominated by agriculture upstream and dense urban development downstream. The results of this study will be compared to findings from a similar investigation recently completed in the coastal waters off southwest Florida and  $\delta^{15}\text{N}$ -derived slopes or FWMF reported in the published literature for other marine systems.

**MP002 An evidence of temporal increase of mercury level in offshore region of Japan using archived open sea cetaceans** T. Irai, Ehime University / Center for Marine Environmental Studies (CMES); T. Isobe, Ehime University / Senior Research Fellow Center, Ehime University / Senior Research Fellow, Ehime University / Center for Marine Environmental Studies; Y. Yasuda, S. Urakami, Ehime University; S. Tanabe, Ehime University / Center for Marine Environmental Studies; S.H. Horai, Tottori University / Department of Regional Environment, Tottori University / Associate Professor; T. Yamada, Y. Tajima, National Museum of Nature and Science, Japan. Mercury cycling in the Pacific Ocean has gained significant attention in recent years, especially with regard to raising mercury emissions from Asia. Recent global modeling suggested potential increasing trend of Hg in North Pacific Intermediate Water (NPIW) due to lateral transport of Hg-rich water masses originated from western Pacific. Uncertainty exists concerning whether increases in anthropogenic emissions over time might have caused increased mercury bioaccumulation in the biota in this region. Marine mammals have the highest potential to accumulate Hg, because of their long life span and higher trophic positions in marine food chains. In this study, we examined accumulation features and temporal trends of trace element levels in the liver and brain of striped dolphin (*Stenella coeruleoalba*) and Melon-headed whale (*Peponocephala electra*) which migrate in the range of temperate and tropical regions by the influence of the warm Kuroshio Current. Stranded or bycaught species have been collected from coastal regions of Japan since 1977 to 2011. These samples after collection were stored under  $-25\text{ }^{\circ}\text{C}$  in the environmental specimen Bank (es-Bank), Ehime University. Forty eight striped dolphin (1977 to 2004) and 69 Melon-headed whale (1982 to 2011) specimens were used for chemical analysis. Hepatic Hg levels ranged from  $6.5 - 1100$  and  $12 - 1240\text{ }\mu\text{g g}^{-1}$  (DW) in striped dolphin and Melon-headed whale, respectively. These Hg levels varied depending on the size of individuals but sex dependence was not found. Multiple linear regression analysis which aimed to correct effect of body size indicated significant increase of total Hg level in striped dolphin ( $p < 0.01$ ) and organic Hg level in Melon-headed whale ( $p < 0.05$ ). This result implied temporal increase of Hg level in offshore regions of Japan. Mercury in the brain of striped dolphin and Melon-headed whale correlated positively with hepatic Hg level, and ranged from  $1.4 - 84$  and  $4.3 - 81\text{ }\mu\text{g g}^{-1}$  (DW), respectively. These levels are comparable to the concentrations that were found to cause various toxic effects in animal experiments suggesting that increasing Hg level in open ocean will possibly induce ecotoxicological effects on cetaceans.

**MP003 Assessing the fate of mercury in animal inoculations: Simultaneous determination of ethylmercury and methylmercury in pig livers and kidneys** J.E. Creswell, Brooks Rand Labs; A. Carter, Brooks Rand Labs / Mercury Group Lead; M. Briscoe, M. Kennard-Mayer, Brooks Rand Labs. There has been significant controversy over the use of the ethylmercury-based preservative thimerosal and, as a precautionary measure, it has been phased out of most childhood vaccines used in the United States and the European Union. In the United States, all new vaccines licensed since 1999

are free of thimerosal as a preservative. The preservative is still common in many vaccines used outside North America and Europe. The mechanism of mercury toxicity is not completely understood. There is still significant interest in better understanding the metabolism and accumulation of mercury in different animal species and in evaluating the safety of thimerosal in currently approved vaccines. Tissue deposition of ethylmercury differs from that of more widely-studied methylmercury, emphasizing the importance of measuring ethylmercury in a variety of species. Because of the toxicity of ethylmercury to humans, it is especially important to measure it in food producing animals, such as livestock and fish, raised for human consumption, including those which may have been injected with thimerosal-containing inoculations. We applied a method of simultaneous determination of ethylmercury and methylmercury to cryogenically-ground pig livers and kidneys by derivatization, purge & trap, gas chromatographic separation, pyrolytic decomposition, and atomic fluorescence spectrometry. Liver and kidney samples were selected as these are organs known to accumulate large percentages of the total mercury burden resulting from thimerosal-containing inoculations. This method quantifies the most prevalent organomercurial species in these organs with a single preparation and analytical run, making it simple and relatively low-cost to perform. We validated the performance of the method by analyzing NIST Standard Reference Material 955c: Toxic Elements in Caprine Blood, as well as matrix spikes into the pig liver and kidney tissues. To evaluate the applicability of the method to other animal species, we also analyzed spiked and unspiked freeze-dried fish tissue samples. We report ethylmercury, methylmercury, and inorganic mercury data from the livers and kidneys of pigs and from freeze-dried fish tissue samples.

**MP004 Biomagnification of mercury in a small polynya Arctic food web** L. Arsenault, Acadia University; N.J. O'Driscoll, Acadia University, Acadia University / Department of Earth & Environmental Sciences; M. Mallory, Acadia University / Canadian Wildlife Service; K. Kidd, University of New Brunswick. Mercury is a contaminant of ongoing concern in the Arctic due to its persistence and impacts on the health and reproduction of biota. Polynyas are productive systems that are critical to the feeding, reproduction, and migration of marine birds and mammals. Small polynyas are common in the Arctic; however, the biomagnification of mercury in these systems has not been studied. We examined the biomagnification of mercury in a small polynya located near Nasaruaalik Island in the Canadian High Arctic. Food web samples including kelp, zooplankton, fish, and chicks were analyzed for methylmercury, total mercury and stable isotopes. Methylmercury was highest in Arctic tern (*Sterna paradisaea*) chicks (liver:  $1.17 \pm 0.90\text{ }\mu\text{g/g}$ , muscle:  $0.69 \pm 0.73\text{ }\mu\text{g/g}$ ), Common eider (*Somateria mollissima borealis*) chicks (liver:  $0.69\text{ }\mu\text{g/g}$ , muscle:  $0.90\text{ }\mu\text{g/g}$ ), and Long-tailed duck (*Clangula hyemalis*) chicks (liver:  $0.48 \pm 0.12\text{ }\mu\text{g/g}$ , muscle:  $0.36 \pm 0.07\text{ }\mu\text{g/g}$ ). Copepods (*Calanus hyperboreus*) had the smallest concentration of methylmercury ( $0.01\text{ }\mu\text{g/g}$ ). Arctic cod (*Boreogadus saida*) and Arctic alligatorfish (*Ulcina olrikii*) had log bioaccumulation factors of 6.09 and 6.29 respectively.

**MP005 Bricks that Breathe: Residential mercury contamination in Huancavelica, Peru** N. Hagan, University of North Carolina at Chapel Hill / Environmental Sciences and Engineering; N. Robins, North Carolina State University / Department of History; H. Hsu-Kim, Duke University / Civil & Environmental Engineering; S. Halabi, Duke University / Department of Biostatistics and Bioinformatics; R. Espinoza Gonzales, Environmental Health Council; D. Richter, Duke University / Nicholas School of the Environment; J. Vandenberg, U.S. Environmental Protection Agency / National Center for Environmental Assessment. Between 1564 and 1810, nearly 17,000 metric tons of mercury (Hg) vapor were released from cinnabar smelting into the atmosphere in Huancavelica, Peru. Following atmospheric transport and transformation, Hg deposited in and contaminated the local community. Ambient soil Hg concentrations are among the highest levels in surface soil in the world, ranging up to  $1200\text{ }\mu\text{g/g}$ . Because residents in Huancavelica have built their modest adobe brick homes using this contaminated soil, concern exists for residential Hg exposure. Problems arise because the interior walls and floors are often unsealed and uncovered, which allows Hg to emanate in both particulate and vapor form. Homes in Huancavelica were sampled for Hg in different media, including adobe bricks, dirt floors, surface dust, and air. Interior adobe brick samples had Hg concentrations as high as  $1670\text{ }\mu\text{g/g}$ , while dirt floor samples ranged up to  $2040\text{ }\mu\text{g/g}$ . Surface dust, likely deposited from resuspended adobe brick and dirt floor particles, contained Hg measuring up to  $660\text{ }\mu\text{g/g}$ . Elemental



Hg vapor concentrations in indoor air were measured up to  $5.51 \mu\text{g}/\text{m}^3$ . The results suggest that there is potentially a significant health risk to the residents of Huancavelica, especially women and children who spend a majority of their time in their homes. Inhalation of particulates and Hg vapor and ingestion of settled particles on food and surfaces potentially cause serious neurological and other health problems. The results of this study will provide a detailed understanding of the relationship of Hg between different sources of Hg within the home, as well as the spatial distribution of Hg across the entire community. A comprehensive analysis of residential Hg contamination and exposure in Huancavelica will guide the development and implementation of mitigation and remediation strategies in the community to reduce health risks from residential Hg exposure. Disclaimer: The information in this abstract has been funded in part by the U.S. Environmental Protection Agency and in part by the EPA/UNC Toxicology Training Agreement CR-83515201-0, with the Curriculum in Toxicology, University of North Carolina at Chapel Hill. It has been subjected to review by the National Center for Environmental Assessment and approved for submission. The views expressed in this abstract are those of the authors and do not necessarily represent the views or policies of the U.S. Environmental Protection Agency.

**MP006 Comparison of Methods Implemented for Quantifying Groundwater Seepage and Mercury Loading into River Surface Water** S. Jelen, V. Onwueme, T. Glover, M. Bystedt, AMEC Environment & Infrastructure. Surface water and groundwater investigations are usually conducted separately when characterizing chemical impacts to water resources during remedial investigations. Hydraulic interconnectivity between groundwater and surface water may be discussed in qualitative terms; however, the locations and amounts of groundwater entering into the river system are rarely quantified *in situ*. The locations and quantities of groundwater seepage into a river system can provide valuable information if groundwater has the potential to serve as an ongoing source of contamination to surface water. In this study, several methods were used to investigate groundwater-surface water interactions along a focused area of a river. The local geology is composed of fractured bedrock, which introduces difficulty in creating accurate mapping of groundwater infiltration using traditional methods, such as seepage meters. The methods selected for this investigation included: 1) infrared imagery along the river bank to identify colder groundwater locations that may be mixing with the warmer surface water, and 2) a real-time specific conductivity survey. Additionally, tracers were added to the river surface water upstream of the study area, and the concentrations of these tracers were measured at defined distances along the study site. The observed changes in tracer concentrations were used to calculate the estimated amount of dilution (i.e., groundwater infiltration) occurring within the study area. The amount of dilution determined from the tracer studies not only provided information on the volume of groundwater entering the river system, but also provided an estimate of chemical loading that was occurring through the combination of tracer data and analytical data for mercury concentrations in groundwater and surface water.

**MP007 Determining rate constants for photochemical reduction of mercury in Arctic snow – Method development and testing** E. Mann, Memorial University of Newfoundland / Environmental Science, University of Toronto Scarborough / Chemistry; N. O'Driscoll, M. Mallory, Acadia University; A. Qureshi, Harvard University; T. Avery, Acadia University; J. Dalziel, R. Tordon, Environment Canada; S. Ziegler, Memorial University of Newfoundland. Photochemical reduction and subsequent volatilization is the primary mechanism for mercury losses from snow prior to the melting. The arctic is a region sensitive to mercury inputs and snow melt may represent an important way for mercury to move into Arctic aquatic ecosystems. To date, the few studies investigating mercury photochemical reduction in snow have been field based and subject to many uncontrolled variables, like temperature and irradiation intensity. The goal of this work was to develop a controlled method to study mercury photochemical reduction in snow, and examine the effects of irradiation intensity. The system consists of a small quartz chamber placed inside a Luzchem photoreaction chamber held at  $-10^\circ\text{C}$ . The Luzchem photoreactor produces UV irradiation intensities similar to early spring and early summer Arctic and temperate natural insolation ( $1.26 - 5.78$  and  $2.44 - 4.99 \text{ W}/\text{m}^2$  in for  $\lambda = 280 - 400 \text{ nm}$ , for the photoreactor and Arctic spring, respectively). Over a 24 hour period mercury-free air effectively strips the sample of  $\text{Hg}(0)$  which is analyzed by cold vapour atomic fluorescence spectroscopy (Tekran 2537A), with a 5 min

resolution. Mercury reduction data were found to fit well with a pseudo first order reaction rate model which was used to quantify reaction rate constants ( $R^2 > 0.98$ ). Triplicate analyses were performed for each radiation intensity, and reduction rate constants were generally within 10% of each other. Using gas phase mercury from a Tekran model 2505 and a Hamilton gas-tight digital syringe, multiple injections into a clean quartz chamber in the Luzchem yielded recoveries of  $98 \pm 2\%$ . With a small sample volume ( $175 - 200 \text{ mL}$  of snow), replicates can be analysed, allowing the generation of confidence limits for the produced reduction rate data. In addition, irradiation conditions can be continually monitored and adjusted to suit the needs of the experiment, allowing for a range of test conditions that are also strictly controlled, which is often not possible with *in situ* studies. This methodology should allow us to generate a diverse set of mercury reduction rate constants that are lacking in the present literature and sorely needed for mercury fate modeling pursuits in polar and temperate regions.

**MP008 Does EPA Method 7471 Accurately Measure Mercury Concentrations in Fish?** T. Ellis, A. Carter, M.S. Um, J. Creswell, M. Briscoe, B. Thorsness, Brooks Rand Labs. We compared the extraction efficiencies of the sample preparation methods described in EPA Methods 7471 and 1631 Appendix for total mercury in fish samples. Many labs use EPA 7471 – “Mercury in Solid or Semisolid Waste (Manual Cold-Vapor Technique)” – for the determination of mercury in biological samples, although it is only approved for “soils, sediments, bottom deposits, and sludge-type materials.” EPA 1631 – “Mercury in Water by Oxidation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry (CVAFS)” – is approved for the analysis of water samples, and has an appendix – “Total Mercury in Tissue, Sludge, Sediment, and Soil by Acid Digestion and  $\text{BrCl}$  Oxidation” – that extends the method to tissue analysis. In a side-by-side analytical comparison on fish samples, we observed that the preparation method from EPA 7471 resulted in mercury concentrations 20% lower than the method from EPA 1631 Appendix, when all sample digestates were analyzed by the same CVAFS technique. The sample preparation procedures described in EPA Methods 7471 and 1631 differ in the oxidation reagent used and the digestion parameters. We hypothesized that incomplete digestion, incomplete mercury oxidation, or a combination of both are the cause of the low recoveries associated with EPA 7471. EPA 7471 specifies a digestion time of 30 minutes and potassium permanganate oxidation, while EPA 1631 Appendix specifies a digestion time of at least four hours and bromine monochloride oxidation. To test for incomplete digestion, we prepared a set of samples and CRMs following the digestion protocol in EPA 7471, but with increased digestion times. To test for incomplete mercury oxidation, we prepared a parallel set of samples and CRMs following EPA 1631 Appendix, but using potassium permanganate as the oxidizing reagent instead of bromine monochloride. We conclude that EPA Method 7471 poses a risk of low-biased data for total mercury in biological tissues.

**MP009 Effect of Natural Water Constituents on the Photo-Decomposition of Methylmercury** M. Kim, Y. Choi, Seoul National University; K. Zoh, Seoul National University, Seoul National University / Department of Environmental Health, School of Public Health. Methylmercury ( $\text{MeHg}$ ) is a highly toxic form of the mercury ( $\text{Hg}$ ) species, and can be accumulated into aquatic food chain. It is reported that  $\text{MeHg}$  in natural water is controlled by biotic and abiotic factors. Although numerous studies have demonstrated that  $\text{MeHg}$  decomposition can be microbially mediated in water and sediment, the photo-induced decomposition of  $\text{MeHg}$  in natural water is known as the another significant pathway to reduce  $\text{MeHg}$  accumulation in water environment. In this study, we investigated the effect of natural water constituents such as  $\text{NO}_3^-$ ,  $\text{Fe}^{3+}$ ,  $\text{HCO}_3^-$ , and dissolved organic matter (DOM) such as humic acid and fulvic acid on the kinetics of  $\text{MeHg}$  photo-decomposition under UVA irradiation ( $\lambda = 365 \text{ nm}$ ). The concentrations of  $\text{NO}_3^-$ ,  $\text{Fe(III)}$ ,  $\text{HCO}_3^-$ , humic acid, and fulvic acid used in the experiment were in the similar ranges found in surface water environment. Photodegradation of  $\text{MeHg}$  under UVA irradiation followed the pseudo-first-order reaction kinetics. Approximately 63% of  $\text{MeHg}$  was decomposed after 90 min during UV photolysis only reaction. Half-life of  $\text{MeHg}$  varied from 3 to 63 min depending on the UVA intensity which ranged from  $0.3$  to  $3.0 \text{ mW cm}^{-2}$ . Interestingly, the photodecomposition rate of  $\text{MeHg}$  increased in the presence of  $\text{NO}_3^-$ ,  $\text{Fe(III)}$ , and fulvic acid due to their photosensitization effect, whereas humic acid and  $\text{HCO}_3^-$  ion inhibited the demethylation rate due to the reduction of light penetration and OH radical scavenging effect. This result clearly support that  $\text{MeHg}$  decomposition

in surface water is induced by UV irradiation and OH radical is a critical factor in the photo-decomposition of MeHg. Also, our result implies that the level of MeHg in natural water can be affected the variation of natural water constituents such as  $\text{NO}_3^-$ ,  $\text{Fe}^{3+}$ ,  $\text{HCO}_3^-$ , humic acid, and fulvic acid concentration along with sunlight intensity.

**MP010 Effect of Organic Matters on the Abiotic Photo-production of Methylmercury in Water** M. Kim, Y. Choi, Seoul National University; K. Zoh, Seoul National University, Seoul National University / Department of Environmental Health, School of Public Health. Mercury is a toxic element found in a variety of natural water systems. Numerous experiments by microbiologists suggest that biotic sediments are major sites for methylation of inorganic mercury and that sulfate-reducing bacteria contribute considerably to methylmercury. However, these processes cannot account for all of the methyl mercury that is formed naturally. Purely chemical methylation of mercury is possible only if suitable methyl donors are present. Chemical reagents thought to cause abiotic methyl mercury formation include small organic molecules such as formate, acetate, malonate and larger organic components of dissolved organic matter such as fulvic and humic acids. In this study, we investigated the abiotic photochemical reaction of Hg(II) with various small organics such as formate, acetate, malonate, and large organics such as fulvic and humic acid in the absence and presence of UVA light. The result showed that the production of MeHg from of (Hg(II)) was proceeded via complexes between Hg(II) and organic matter. The production of MeHg was significantly enhanced by the presence of UVA light compared to dark condition. MeHg production rate with the irradiation of UVA when acetate is present was highest among the organics. MeHg production rate in the presence of UVA increased as the increase in acetate concentration, whereas MeHg production decreased as the increase of acetate concentration in the dark condition. The production of MeHg from Hg(II) with acetate was much higher than that by Hg(II) with formate, and malonate, indicating acetate is a suitable methyl donor. The presence of fulvic and humic acid also production MeHg from Hg(II) ion in the presence of UVA light, but the MeHg productions were small compared to when small organics were present. Our result implies that abiotic MeHg production can be mediated by sunlight in the presence of Hg(II) and suitable organic matters as methyl donors in natural water.

**MP011 EPA's Assessment of Mercury in Fish from U.S. Rivers** L.L. Stahl, J.B. Wathen, US EPA, Office of Water / Office of Science and Technology; A.R. Olsen, U.S. EPA, Office of Research and Development / Western Ecology Division, National Health and Environmental Effects Laboratory; B.D. Snyder, Tetra Tech, Inc. / Center for Ecological Sciences; H.B. McCarty, CSC. Elevated mercury concentrations in fish are the leading cause of fish consumption advisories. In a continuing effort to characterize the extent of mercury contamination in the Nation's waters, EPA's Office of Water and Office of Research and Development collaborated to conduct the first statistically-based survey of mercury in fish from U.S. rivers. This national fish survey was conducted under the framework of EPA's National Rivers and Streams Assessment (NRSA), a probability-based survey designed to assess the condition of the Nation's streams and rivers. In 2008 and 2009, field teams applied consistent methods nationwide to collect samples of fish commonly consumed by humans at 566 randomly selected river locations ( $\geq 5$ th order based on 1:100,000-scale Strahler order) in the lower 48 states during June through October. They collected one fish composite sample at every sampling location, and each composite sample consisted of five similarly-sized adult fish of the same species. Largemouth and smallmouth bass were the primary species collected for the study, accounting for 34% and 24% of all fish composites, respectively. Fillets from the fish composite samples were analyzed for total mercury using a Direct Mercury Analyzer. Study data show that mercury was detected at quantifiable levels in every fish sample collected for the study (quantitation limits ranged from 0.5-1.5 ng/g or ppb). Based on results from this probabilistic study, the mean concentration of mercury in fish from U. S rivers is 231 ppb, and the maximum mercury concentration measured for the fillet samples is 1,419 ppb. Nearly 26% (or 17,775 river miles) of the total 69,432 river miles included in this survey had mercury fish tissue concentrations exceeded EPA's 300 ppb human health screening value.

**MP012 Experimental Control of Methylmercury in Rice Wetlands using Alternative Rice Harvest Practices** C. Eagles-Smith, US Geological Survey / FRESC; J.T. Ackerman, USGS-WERC; J. Fleck, L. Windham-Myers,

US Geological Survey; H. McQuillen, Bureau of Land Management; W. Heim, Moss Landing Marine Laboratory; M. Stephenson, California Department of Fish and Game. Mercury (Hg) threatens ecosystem health at a global scale, yet Hg risk can differ substantially among habitat types because of habitat-specific variations in the biogeochemical, hydrological, and ecological factors that regulate the production and bioaccumulation of methylmercury (MeHg). Wetlands often are associated with elevated MeHg concentrations, and agricultural wetlands such as rice fields may support particularly high MeHg production. Rice fields are characterized by a series of extreme wetting and drying cycles, sulfate-containing fertilizers, and high levels of labile organic carbon, which are all key components to the Hg cycle. Importantly, rice fields comprise approximately one-fifth of freshwater habitats and 11% of cultivated land area globally, they provide critical wildlife habitat, and offer substantial economic, human health, and ecosystem benefits. Thus, there is strong impetus to better understand the drivers of Hg cycling in rice fields and develop useful management approaches to mitigate Hg risk associated with rice without compromising productivity. We employed a series of replicated post-harvest experiments at a production-agriculture scale to test the applicability of implementing low-cost management techniques to alter carbon cycling as a way to control MeHg cycling. Specifically, after harvest (and prior to post-harvest flooding) we either: (1) tilled residual rice straw (carbon) into the soil, (2) bailed rice straw and removed bails from fields, or (3) followed standard practices of leaving straw in the fields for natural decomposition. We measured the response to our treatments at the inlets and outlets of each wetland during the following winter and subsequent growing season using an integrated suite of indicators. We employed caged fish as bioindicators to evaluate MeHg bioaccumulation over discrete timeframes, and monitored temporal fluctuations in aqueous carbon and MeHg dynamics at the same locations. Our preliminary results indicate that both caged fish and aqueous Hg concentrations were elevated in rice fields relative to non-agricultural wetlands with similar hydrological management. However, our management techniques resulted in a wide gradient in available carbon across rice fields. Moreover, we found that aqueous MeHg concentrations were positively correlated with the biomass of surface litter across rice fields, suggesting that that carbon management may influence MeHg cycling in these managed systems.

**MP013 Factors Limiting Mercury Bioaccumulation in Contaminated Estuarine Systems** B. Henry, Exponent; D. Glaser, Anchor QEA, LLC; S. Brown, Rohm and Haas Chemicals LLC / Department of Toxicology, The Dow Chemical Company / Global Remediation. Salt marshes are considered potential sources of methylmercury to the coastal pelagic zone and to marsh biota. Methylation rates and methylmercury concentrations in pore water have been reported as negatively correlated with pore water sulfide and positively correlated with some measures of dissolved organic matter. Extrapolation of this conceptual model of mercury behavior to marshes in which total mercury levels are elevated due to local sources, however, is problematic. Marshes with elevated total mercury in sediments do not necessarily exhibit proportionally elevated concentrations of methylmercury in sediment pore water and resident biota. Based on literature review, we have developed a conceptual model to explain the disconnect or lack of correlation sometimes observed between elevated total mercury and methylmercury in tidal marshes subject to local sources of mercury, and thus the relative insensitivity of some estuarine systems to mercury contamination. Key factors potentially include 1) food webs with strong ties to the water column; 2) a large volume of tidal exchange that reduces mercury concentrations in surface water, 3) high concentrations of sulfide that limit methylation in sediment, 4) high organic carbon content that results in sequestration of mercury and reduced availability of inorganic mercury for methylation, and 4) extensive movement of crabs and larger fish throughout estuaries, which limits local exposure. These factors may interact to limit mercury bioaccumulation in contaminated estuarine systems (i.e., compared to that expected based on total mercury concentrations in sediment). The lack of correlation between total mercury in sediment and methylmercury exposure to biota has important implications for risk assessment and the determination of remedial approaches. Data from a contaminated tidal marsh system will be used to illustrate the model.

**MP014 Impact of salt marsh reclamation on mercury fate and biogeochemistry** A. Godfrey, Acadia University / Earth and Environmental Sciences; T. Sizmur, Iowa State University / Cademartini Lab; N.J. O'Driscoll, Acadia University, Acadia University / Department of Earth



& Environmental Sciences. Much of the world's coastline has been dyked to increase the area of arable farmland and eliminate seawater inundation. Efforts are now being made in some areas to intentionally breach these dykes and restore the land and re-create the original salt marsh habitat. Salt marshes accumulate sediments over time and build up natural levees that protect the coastline against storm surges and, potentially, against sea level rise. We investigated one such reclamation site (Beaubassin, New Brunswick, Canada) that was breached in October of 2010. Sediment cores were taken from (i) behind a dyke ten months after it was intentionally breached, (ii) a neighbouring salt marsh, and (iii) the mudflats on the seaward side of the dyke. These samples were compared with cores collected the previous summer, before the dyke had been breached. Cores were sectioned, dried, and the solids analyzed for both total mercury (THg; using thermal degradation AAS) and methyl mercury (MeHg; using ethylation, tenax trap, and GC-AFS). Total mercury concentrations in the post-breach cores were approximately double the concentrations in the pre-breach cores, throughout the depth profile. All cores showed THg decreasing with depth, except the salt marsh which revealed similar THg concentrations down the profile. Mean THg sediment concentrations in the upper 2 cm of the cores were  $10.64 \pm 0.48 \text{ ng g}^{-1}$  (pre-breach),  $12.35 \pm 2.67 \text{ ng g}^{-1}$  (mudflat),  $20.23 \pm 1.68 \text{ ng g}^{-1}$  (post-breach), and  $20.28 \pm 3.92 \text{ ng g}^{-1}$  (salt marsh). The post-breach cores contained the greatest concentrations of MeHg in the upper 2 cm of sediment ( $0.57 \pm 0.14 \text{ ng g}^{-1}$ ), decreasing with depth. Pre-breach cores showed the opposite trend with the lowest concentrations in the upper core ( $0.36 \pm 0.02 \text{ ng g}^{-1}$ ) and an increase with depth. As a percentage of THg however, the MeHg concentration was lower pre-breach (3.41%) than post-breach (2.79%). Methylmercury in the post-breach cores and the salt marsh cores were similarly distributed as they both contained elevated MeHg in the upper core. The mudflat cores contained the lowest MeHg concentrations. Salt marsh restoration may increase the availability of THg, and possibly MeHg, to coastal ecosystems.

**MP015 Investigating mercury fractionation in water, sediments, and fish from the Husky Lakes, Northwest Territories, Canada** N. Gantner, University of Victoria / Department of Geography; S. McFadyen, University of Victoria; J. Gareis, Aurora Research Institute; J. Reist, Freshwater Institute, Fisheries and Oceans Canada; H. Hintelmann, Trent University / Chemistry. Mercury can accumulate in apex-predator fish muscle to concentrations exceeding those considered safe for subsistence consumption by humans. Fish species such as Lake trout are typical apex-predators of Arctic lakes and can be a significant source of food for local indigenous peoples. The influence of abiotic factors and biological parameters on Hg accumulation in apex-predators are not well understood. Further, a good understanding of sources of Hg to and processes within water column and food webs is still lacking. Our study aims to investigate the interactions of water column, food webs and Hg transfer in four freshwater systems in the Inuvialuit Settlement Region (Canada). The selected Big, Yaya, Noell, and Husky Lakes systems represent a range of water column and ecological characteristics, as well as Hg delivery (marine-, riverine- or freshwater-derived). We investigate how those characteristics affect Hg transfer and fractionation. All lakes are frequented by the Inuvialuit communities Inuvik and Tuktoyaktuk for subsistence fishing. Sampling includes surface water, benthic and pelagic invertebrates, tissues from harvested fishes, and non-target fishes. Biological parameters of fishes (age, length, weight, diet) are recorded and invertebrates separated by species. Sample analysis includes total Hg (THg), monomethyl Hg (MeHg), and stable isotopes of carbon ( $\delta^{13}\text{C}$ ), nitrogen ( $\delta^{15}\text{N}$ ), and Hg ( $\delta^x\text{Hg}$ ) and otolith microchemistry. Hg IRs are analyzed by multi-collector inductively coupled plasma mass spectrometry (MC-ICP/MS). Hg mass independent fractionation (MIF;  $\Delta^{199}\text{Hg}$ ) and mass dependent fractionation (MDF;  $\delta^{202}\text{Hg}$ ) will be calculated and evaluated against conditions in the water column, food web transfer and the potentially difference in Hg delivery. [THg] detected in harvested fishes will be compared to consumption guidelines. We will present an overview of this multidisciplinary study and discuss our preliminary findings with particular focus on implications for future research efforts in a changing Arctic environment.

**MP016 Investigation of mercury methylation pathways in biofilm versus planktonic cultures of *Desulfovibrio desulfuricans*** T. Lin, UCLA / Civil and Environmental Engineering; R. Kampalath, C. Lin, M. Zhang, K. Chavarria, J. Lacson, J. Jay, UCLA. Biofilms can methylate mercury at higher rates than unattached bacteria and are increasingly recognized as important mercury methylation sites in the environment. Our previous

study showed that methylation rates in biofilm cultures were up to an order of magnitude greater than those in planktonic cultures of a sulfate-reducing bacterium. To probe whether the differential methylation rates resulted from metabolic differences between these two cultures, mercury methylation assays following molybdate or chloroform inhibition (a specific inhibitor of the acetyl-CoA pathway) were conducted on biofilm and planktonic cultures of *Desulfovibrio desulfuricans* strains M8 and ND132. Molybdate was as effective in inhibiting mercury methylation in both planktonic and biofilm cultures as in inhibiting growth. Unlike planktonic cultures, addition of chloroform only impacted mercury methylation in biofilm cultures, indicating a role for the acetyl-CoA pathway. Expression of the *cooS* gene, encoding for carbon monoxide dehydrogenase, a key enzyme in this pathway, was compared in biofilm and planktonic cultures of ND132. Biofilm cultures showed up to 4 times higher expression of *cooS* than planktonic cultures. Although planktonic cultures of *D. desulfuricans* did not methylate mercury as significantly via the acetyl-CoA pathway, in biofilm cultures the acetyl Co-A pathway was expressed at a higher rate and appeared to be employed in mercury methylation in biofilm cultures. This observation has important implications, particularly in developing reliable models to predict mercury methylation rates in different environments and perhaps eventually in being able to control this undesirable chemical transformation.

**MP017 Mercury in breeding common loons, lake sediments and prey fish, and potential effects on health of loons in Quebec, Canada** L. Champoux, Environment Canada / SCIENCE AND TECHNOLOGY BRANCH; D. Masse, La Mauricie National Park, Parks Canada; A. Scheuhammer, Environment Canada / SCIENCE AND TECHNOLOGY BRANCH. As part of a national program assessing the ecological risks of mercury to freshwater fish and wildlife across Canada, a study was conducted to determine environmental and biological factors that best explain variability in Hg levels in fish and loons across study sites in Quebec, Canada. We also assessed Hg concentrations in fish and loons in respect to Hg toxicity. In Quebec, from 2008 to 2010, loon reproductive success was assessed on 15 lakes. Blood and feathers were collected for total mercury and various biomarkers were assessed. Sediments and prey fish samples were also collected for Hg analysis. Stable N and C isotopes were measured in loon blood, fish muscle and benthos in order to take into account the food source and trophic position across lakes. Small fish from some lakes had Hg concentrations sufficiently high to pose a health risk to common loons or other fish-eating wildlife; 17% of the loons sampled (23% of the adults and 12% of the juveniles) had blood Hg concentrations sufficiently high to pose a risk to health. Relationships between levels of thyroid hormones, steroid hormones and cholinesterase and Hg concentrations were determined.

**MP018 Mercury in Mecon river and Foster Bay (Deception Island, Antarctica)** J. Canario, IPMA IP / Aquatic Environment Department, IPMA I.P. / Aquatic Environment and Biodiversity, INRB IP/IPIMAR / Aquatic Environment and Biodiversity; A. Mao de Ferro, A. Mota, Instituto Superior Técnico. Mercury (Hg) is pollutant of great environmental concern mainly due to the well known toxicity of its compounds, particularly its methylated form. Atmospheric transport is the main dispersion mechanism for mercury and is the reason why this contaminant is often found in relatively high concentrations far from emission sources. These are the case of the Polar Regions where relatively high levels of mercury compounds were found in sediments, water and particularly biota. While in the Arctic very much is known about the biogeochemical cycle of Hg, in the Antarctic the behaviour of Hg is less understood. In December 2011 a field work took place in Deception Island, a volcanic island in the South Shetland Archipelago, Antarctica. Sediments, ice, water and suspended particulate matter were collected in Mecon River and in the Foster Bay during 8 consecutive days. All samples were analysed for total mercury (particulate -  $\text{Hg}_p$  - and dissolved  $\text{Hg}_d$ ), methylmercury (MeHg) and other interpretative physico-chemical parameters. Total mercury concentrations in the upstream stations of Mecon River were lower compared to the downstream ones and Foster Bay. Dissolved Hg levels varied from 0.54 ng/L in station #3 to 2.36 ng/L in the Bay and particulate Hg concentrations from 0.012  $\mu\text{g/g}$  to 1.60  $\mu\text{g/g}$  at the same sample sites. In sediments, concentrations were similar in the River ( $0.096 \pm 0.022 \mu\text{g/g}$ ) sites but much higher in Foster Bay ( $0.845 \pm 0.422 \mu\text{g/g}$ ). Methylmercury concentrations were below DL in the river and account less than 2.5% of total Hg in the Foster Bay. Time course evolution showed that Foster Bay is the main source of Hg to the downstream river waters. This aspect was particularly evident during flood tide. Moreover, Hg levels



determined during this campaign in active volcanic vents near the bay suggest that these active volcanic sites could be the main Hg source to this ecosystem.

**MP019 Mercury speciation and bioaccessibility from adobe bricks and dirt floors in Huancavelica, Peru** N. Hagan, University of North Carolina at Chapel Hill / Environmental Sciences and Engineering; N. Robins, North Carolina State University / Department of History; H. Hsu-Kim, Duke University / Department of Civil and Environmental Engineering; J. Vandenberg, U.S. Environmental Protection Agency / National Center for Environmental Assessment; D. Leith, University of North Carolina at Chapel Hill / Environmental Sciences and Engineering. Huancavelica is located in the poorest district of Peru where its residents are faced with living with over 400 years of mercury (Hg) contamination. Many of the residents are not aware of the high levels of Hg contamination throughout the city, and those who are informed lack the resources to alleviate the problem. Ambient soil concentrations of total Hg in Huancavelica range up to 1200 µg/g, among the highest concentrations measured in surface soil in the world. Because the residents of Huancavelica continue to build their modest homes from adobe brick formed from this contaminated soil, concern exists for residential Hg exposure. Concentrations of total Hg in adobe bricks and dirt floors have been measured up to 1670 µg/g and 2040 µg/g, respectively. Sequential selective extractions were used to quantify the amount of Hg in the sample that is water soluble, human stomach acid soluble, organo-chelated, elemental Hg, and mercuric sulfide. Simulated gastric fluid and phagolysosomal fluid extractions were used to estimate the bioaccessibility of Hg from ingestion or inhalation of Hg-contaminated particles within the home. The results of this study will provide a detailed understanding of the potential risks from exposure to Hg in adobe bricks and dirt floors in homes in Huancavelica, and will yield the fundamental knowledge to identify and evaluate effective mitigation strategies to seal adobe brick walls and dirt floors in homes where the greatest risk of exposure to Hg is present. The application of the sealant may ultimately reduce residential exposure to Hg contamination in Huancavelica, Peru. Disclaimer: The information in this abstract has been funded in part by the U.S. Environmental Protection Agency and in part by the EPA/UNC Toxicology Training Agreement CR-83515201-0, with the Curriculum in Toxicology, University of North Carolina at Chapel Hill. It has been subjected to review by the National Center for Environmental Assessment and approved for submission. The views expressed in this abstract are those of the authors and do not necessarily represent the views or policies of the U.S. Environmental Protection Agency.

**MP021 Quantifying Mercury Biomagnification Using Trophic Magnification Factors** J. Williams, TerraGraphics Environmental Engineering. Trophic magnification factors (TMFs) are frequently used to quantify mercury biomagnification rates in aquatic food webs. TMFs are typically calculated as the slope of a regression between organism Hg and  $\delta^{15}N$  or trophic level ( $\log_{10}Hg = a + b\delta^{15}N$ , where  $TMF=10^b$ ). Mercury TMFs are often noted to be remarkably constant across studies, but variation in study design can affect the comparability of TMFs. A literature review was performed to assess the comparability of TMF studies and identify trends in TMF data across systems. 178 reported mercury TMFs were identified among 47 studies. TMFs were reported for freshwater lentic (52% of TMFs), freshwater lotic (20%) and marine systems (28%) and values of  $b$  ranged from 0.08 to 0.53 for  $CH_3Hg$  TMFs and -0.28 to 0.66 for total Hg TMFs. The comparability of TMFs will be described with respect to study design and methodology. The types of organisms included in TMF regressions (whole food web or only fish), use of stable isotope mixing space screening, use of  $CH_3Hg$  or total Hg data, use of wet or dry weight mercury data, and other factors can affect the representativeness and comparability of TMFs. Patterns in TMF data associated with study design, ecosystem type, food chain length, and mercury concentrations at the base of the food web will be detailed.

**MP022 Seasonal variation of mercury diffusive fluxes in the sediment/water interface in the Tagus Estuary (Portugal)** R. Cesario, INRB-IPIMAR National Institute of Biological Resources / Department of Environmental and Biodiversity; J. Canario, IPMA IP / Aquatic Environment Department, IPMA I.P. / Aquatic Environment and Biodiversity, INRB IP/IPIMAR / Aquatic Environment and Biodiversity; A. Mota, Instituto Superior Técnico; M. Caetano, IPMA I.P.; C. Monteiro, Instituto Superior Técnico; M. Nogueira, C. Vale, IPMA I.P. Mercury (Hg) in the

environment constitutes a threat to aquatic life and human health, especially in its methylated form (MeHg). Among other transport mechanisms molecular diffusion play an important role being responsible for the increase of mercury concentrations in the water column due to the Hg concentrations gradient in pore- and over-lying waters. In this work we hypothesize that mercury and methylmercury diffusive fluxes may vary seasonally due to the well known seasonal variation of methylmercury concentrations in surface sediments of the Tagus Estuary. In order to confirm this hypothesis sediment cores and overlying waters were collected in summer of 2010 and winter of 2010/2011. Sediments were sliced in 1-2 cm and pore-waters were separated. Solid sediments were analyzed for Hg, MeHg. Dissolved reactive and total mercury ( $Hg_R$  and  $Hg_T$ ) as well as MeHg were quantified in pore- and overlying waters. No significant differences were observed for total mercury concentrations in solid sediments in both periods. Otherwise, in summer MeHg levels increase significantly (up 23 times) in both dissolved and solid fractions. These results may be explained by the increase of available  $Hg_R$ , dissolved organic carbon and bacterial activity and the lower redox potential. Inorganic mercury ( $Hg-Cl_x^{n-}$ ), Hg-Natural Organic matter and MeHg molecular diffusive fluxes were estimated using pore- and overlying-water Hg gradients. Downward (from water to sediment) inorganic mercury fluxes were found in #ALCX and #CN in summer and in #BRR at winter period. A different pattern was observed for MeHg with downward fluxes in summer period and upward fluxes in winter period, in all sites. The opposite trend found between summer and winter fluxes could be explained by different extension of MeHg demethylation in the water column.

**MP023 Graphical Excellence: Considerations when Presenting Data** W. Swanson, Neptune and Company, Inc, Neptune & Company, Inc.. There is more than one way to make a plot. When presenting data in a scientific report or journal, the objective should be to use plots that make interpretation easy, without distortion. "Graphical excellence consists of complex ideas communicated with clarity, precision and efficiency. (It conveys to) the viewer the greatest number of ideas in the shortest time with the least ink in the smallest space." (Tufte, 1983). What is best way to summarize data results from soils-related studies? How can the environmental fate and effect of soil contaminants be presented in plots in a risk assessment? Some general plot types are presented and examples provided to demonstrate which presentation style is most appropriate for a given situation. The choice of plot will depend on the type and complexity of the data and the nature of the summary or relationship to be portrayed. A plot that works for one data set may be a bad choice for another data set. Examples of good plots and bad plots are taken from literature and soils-related consulting projects to illustrate the basic ideas.

**MP024 A Novel Approach for Estimating Soil Exposure Point Concentrations in Ecological Risk Assessment** B. Anthony, W. Stiteler, M. Erickson, ARCADIS U.S., Inc; G. Griffith, Georgia-Pacific LLC. One of the primary uncertainties with exposure modeling in Ecological Risk Assessment is estimating the appropriate spatial scale for developing exposure point concentrations (EPCs) for the identified receptors. Terrestrial exposure areas within a given site can be estimated based on receptor foraging ranges by assuming that ranges are adjacent and do not overlap. However, this is often not consistent with receptor behavior and the specific location of any given foraging range within a larger site is unknown. An alternative approach is to calculate an average EPC for the total site area, even when it is larger than the estimated foraging range for an individual, as a means to address potential population-level risks. Both approaches are subject to considerable uncertainty with respect to individual receptor exposure and population-level risks. To address these uncertainties at a site with PCB-impacted floodplain soils, a "moving window" method was used in conjunction with spatially interpolated surface soil PCB concentrations in a terrestrial Baseline Ecological Risk Assessment (BERA) conducted for the Kalamazoo River Superfund Site in Michigan. Spatial-interpolation provided a means to incorporate a large number of biased sample points together with unbiased samples. Based on the suite of receptors evaluated in the BERA, 1-acre, 2-acre and 11-acre circular moving windows were evaluated on a one-foot grid over the spatially interpolated surface. This resulted in the EPC surface being represented by an exposure value at 1-foot grid points across the entire floodplain, supporting expression of the BERA hazard quotient results as a percent of foraging ranges potentially affected. This type of output may allow for better-informed risk management decisions for protection of local populations of receptors.

**MP025 Impact of initial concentration of diesel oil on the microbial adaptive responses to phenanthrene and benzo[a]pyrene mineralization**

O.O. Igunnugbemi, K.T. Semple, Lancaster University / Lancaster Environment Centre. The impact of initial diesel oil concentration on microbial adaptive responses to further pollution incidents occurring in previously exposed soil was investigated by monitoring the development of indigenous catabolism of a model low molecular weight polycyclic aromatic hydrocarbon (LMW PAH – phenanthrene) or high molecular weight polycyclic aromatic hydrocarbon (HMW PAH – benzo[a]pyrene). Radiorespiratory assay was used to assess  $^{14}\text{C}$ -PHE or  $^{14}\text{C}$ -BaP catabolism in four UK soils with distinct physicochemical characteristics. Soils previously exposed to diesel oil at 500 or 5000 mg kg<sup>-1</sup> for 30 or 150 d were further amended with diesel oil at 500 mg kg<sup>-1</sup> and microbial degradation of PHE and BaP monitored until  $^{14}\text{CO}_2$  evolution plateaued. The initial concentration of diesel oil significantly affected dissipation of HMW PAHs upon further exposure to diesel oil, but did not have appreciable effect on LMW PAHs. However, multivariate analysis indicated that soil organic C contents and residence time played major role in development of BaP catabolism in the soils pre-exposed to low concentration of diesel oil. We propose that induction of BaP biodegradation is a function of (i) an initial moderate concentration of diesel oil, (ii) duration of exposure before recontamination and (iii) soil physical-chemical characteristics. These findings indicate that the impact of initial concentration of diesel oil on the development of HMW PAH catabolic induction could be influenced by soil organic matter and duration of exposure.

**MP026 Preliminary Ecotoxicological Assessment of Plant Based Biodiesels Compared to Diesel**

I. Bamgbose, Texas Tech University / The Institute of Environmental and Human Health (Department of Environmental Toxicology), Texas Tech University; T.A. Anderson, Texas Tech University / Environmental Toxicology. There has been an increased interest in the use of biodiesel as a potential fuel and a feasible alternative to Diesel fuel. Biodiesels are clean burning alternative fuels produced from livestock feeds and various vegetable oils. In theory, these animal/plant derived materials should have less environmental impact in soil based on their simplified composition relative to Diesel. However, like Diesel fuel, biodiesel fuel could accidentally be spilled into aquatic and terrestrial environments. Consequently, their potential impacts on organisms should be evaluated. In this study, the effects of soil contaminated with Diesel and two plant-derived Biodiesels (safflower methyl-ester and castor ethyl-ester) were evaluated using several ecotoxicological tests including, an earthworm (*Eisenia fetida*) 14 day lethality and reproduction assay, an earthworm filter paper contact test, and seed germination assays. In the seed germination assays, four plant species were used in order to investigate germination and survive in a gradient of fuel-contaminated soil: alfalfa (*Medicago sativa*), lettuce (*Lactuca sativa*), radish (*Raphanus sativus*) and wheatgrass (*Triticum aestivum*). Of the plants tested, lettuce seeds showed the most sensitivity to Diesel, Castor biodiesel, and Safflower biodiesel contaminated soils (silt and sandy loam); most of the lowest germination rates were for lettuce seeds. Like lettuce, wheatgrass was also sensitive to the three fuels with the most sensitivity to Diesel-contaminated soils. Radish seeds, which typically germinated very rapidly (within 4 days), showed the least sensitivity to all three fuels in both soil types. Alfalfa also showed some germination inhibition; however, the interaction between the fuel and soil appeared to be a factor in alfalfa germination success. Both Castor ethyl ester and Safflower methyl ester can have effects on the germination of crop plants. These results are some of the only data available on biodiesel effects on soil systems in general and seed germination in particular.

**MP027 Risk Assessment of petroleum hydrocarbon contamination in soil. I. Migration of conventional and renewable fuels in accidental spill scenarios**

V. Malk, University of Helsinki / Palmenia Centre for Continuing Education; E. Barreto Tejera, Autonomous University of Madrid / Department of Biology; S. Simpanen, University of Helsinki / Department of Environmental Sciences; J. Hakkinen, University of Turku / Centre for Maritime Studies; R. Makela, A. Kiiski, University of Helsinki / Palmenia Centre for Continuing Education; O. Penttinen, University of Helsinki / Department of Environmental Sciences. Frequent disasters in aquatic and terrestrial environments have caused researchers and authorities to realize the potential ecotoxicological threat that chemical accidents can pose to the environment. Most common chemical spills concern oil products, such as diesel oil or gasoline. When a PHC spill occurs in soil, there are many chemical specific, biogeochemical, and temporal variables that dictate how

contaminant moves through environment. Oils typically migrate in soil as non-aqueous phase liquids (NAPL). Computer models and calculations are widely used in risk assessment to estimate distribution of chemicals in contaminated sites, but calculated values should always be carefully used to interpret the real nature. In this study migration of conventional gasoline and diesel and their most attractive substitutes in Finland, renewable diesel NExBTL and ethanol blended gasoline RE85 were investigated on a laboratory scale by dyeing fuels and monitoring their migration in dry and wet quartz sand in a transparent tank. Results from laboratory experiment were compared to values calculated with commonly used analytical equations. Furthermore the real accident case data was compared to values modeled with Hazardous Materials Transportation Environmental Consequence Model (HMTECM). Calculated maximum migration depths of different fuels in dry soil were 120 to 180 % from experimental depths determined in the laboratory experiment. In the real accident case migration depth modeled with HMTECM model was 80-150 % from the measured migration depth. Results showed that calculations can give suggestive estimation about the migration of fuels in the NAPL phase in worst case accident scenario. There was no significant difference in migration of conventional diesel and NExBTL in the laboratory experiment, but RE85 migrated 50 % slower in sand as NAPL phase than normal gasoline.

**MP028 Assessment of the Effects of Phenanthrene and its Nitrogen Heterocyclic Analogues on Microbial Activity in Soil**

I.N. Anyanwu, / Lancaster Environment Centre; K.T. Semple, University Lancaster / Lancaster Environment Centre. The effects of phenanthrene and its nitrogen heterocyclic aromatic (NHAs) analogues on soil microbial respiration were evaluated to assess the toxicity of the chemicals in soil using substrate induced respiration (SIR). Soil samples were amended with phenanthrene and its nitrogen containing analogues and respiration rates were measured over time. The results showed that inhibition of PAH amended soil plateaued after 30 days, while the nitrogen containing analogues showed a pattern of respiration inhibition/stress with increased concentration and aging. The time dependent percentage inhibition/stress was more than 25% which portrays NHAs toxicity. Also, ANOVA results showed significant and strong positive correlation between mean  $\text{CO}_2$  evolved and incubation days (aging). The estimated LOEC was 10 mg/kg based on the concentrations used. This suggests that soil micro-organisms might be more sensitive to NHAs in soil than the homologues PAHs.

**MP029 Do metal impacts in soil alter mammalian bioavailability of polycyclic aromatic hydrocarbons (PAH) in incidentally ingested soil?**

K. James, University of Saskatchewan / Soil Science, University of Saskatchewan / Toxicology Centre; S.D. Siciliano, University of Saskatchewan / Toxicology Group; M. Cave, British Geological Survey; P. Mayer, Aarhus University / National Environmental Research Institute, Aarhus University / Department of Environmental Science, National Environmental Research Inst. / Environmental Chemistry & Microbiology; R. Peters, University of Saskatchewan / Department of Soil Science. The majority of contaminated sites contain elevated levels of metals and hydrocarbons. Yet, risk assessments of either metals or hydrocarbons typically do not consider how these two classes of pollutants may influence exposure to human or ecological receptors. Here, we evaluated the hypothesis that metal concentrations in soil altered mammalian PAH bioavailability. Twenty different contaminated soils with elevated PAH concentrations from North America and Europe, from a variety of sites were used. Using the juvenile swine model, we assessed PAH blood concentrations over a 48 hour time period in six replicate swine which had ingested a 5 g bolus dose of contaminated soil. Using Area Under the Curve analysis, we calculated bioavailability of this ingested soil and compared the bioavailability of these ingested soils to the oral bioavailability of pure PAHs. As expected absolute bioavailability of PAHs in soil was much lower than the current default of 100%. Despite this low bioavailability, we were able to monitor PAH time courses in juvenile swine across soil types. Preliminary evidence suggests that specific metals are influencing mammalian bioavailability. The impact of this on how to assess and derive standards for co-contaminated sites will be discussed.

**MP030 Simulated Earthworm Gut Assessment of Multi-Metal Bioavailability in Petroleum Impacted Soils**

R. Nhan, S.D. Siciliano, University of Saskatchewan / Toxicology Group. Existing technologies assessing the toxicity of contaminated soils that require live organisms are both time and resource intensive. However, this testing is essential in the field of risk

assessment in order to determine client's liability for soil pollution and potential remediation costs. The novel development of an in-vitro laboratory testing technique called the Simulated Earthworm Gut (SEG) has many potential benefits over current methods of determining contamination levels. The SEG is based on the digestive processes of earthworms, where a synthetic earthworm digestive fluid is prepared and undergo testing methods that replicate the digestion and gut content transit time of earthworms. Ultimately resulting in samples used to determine the amount of contaminant absorbed into the organism by advanced laboratory analytical techniques. When this data is used in conjunction with current methods, faster, more cost effective and accurate data can be obtained so less weight can be put on expensive, time consuming live organism tests. Earthworm tests are an important indicator of ecological risks and overall soil quality as they are one of the pioneer soil organisms that create an environment where other vital soil organisms can thrive. Currently, the most effective formulations of the SEG fluid have not been determined. Ongoing work has resulted in promising data in both petroleum hydrocarbon (PHC) and heavy metal contaminant bioavailability but with two different solutions. The goal is to refine and produce a realistic and reliable SEG formulation to test for a wider variety of contaminants. In this pilot study, the formulation used in past PHC experiments will be tested for efficacy in heavy metal contamination. The new SEG formulation accurately predicted metal uptake by earthworms and also allows one to assess hydrocarbon uptake into earthworms. Next steps are to correlate metal toxicity with invertebrate toxicity tests run on these same soils.

**MP031 Evaluating the impact of soil type, ageing, and Zn form on toxicity to *Eisenia fetida* using ZnCl<sub>2</sub>, ZnO bulk powder and ZnO nanoparticles** M. Schulze, Towson University / Urban Environmental Biogeochemistry Laboratory; R. Casey, D.R. Ownby, Towson University / Urban Environmental Biogeochemistry Laboratory, Towson University / Department of Chemistry; S.M. Lev, Towson University / Urban Environmental Biogeochemistry Laboratory, Towson University / Professor. The increasing use of zinc oxide nanoparticles (ZnO NPs) in consumer products and the fate of these particles once they enter the wastewater system requires a risk assessment of these particles in aquatic and terrestrial systems. Of critical importance is an understanding of how ZnO nanoparticles behave once introduced into the soil environment, their potential to induce toxic effects and bioaccumulate in soil dwelling organisms enabling trophic transfer. To date, there is limited information on the long-term behavior of NPs in terrestrial systems. Earthworms are an ideal organism to evaluate the fate and effects of NPs in a terrestrial system since they directly process soil through their digestive system making them susceptible to changes in the chemistry of the soil environment. As they are an important food source for other terrestrial organisms, earthworms may represent a critical entry point into the food web for trace metal pollutants, including NPs, in terrestrial systems. This investigation is part of a larger study to determine the uptake and elimination rates of isotopically-enriched (<sup>68</sup>Zn) ZnO NPs, bulk ZnO powder and ZnCl<sub>2</sub> by *Eisenia fetida* over the course of a 21 day uptake and 21 day elimination period. Earthworms were exposed to two different clean experimental soils (OECD and Potting Soil) for 42 days to evaluate survivability under experimental conditions. Earthworms exhibited weight loss (-0.03 +/- 0.02 g/worm) and lower survival rates (67%) in OECD soil as compared to weight gain (+0.13 +/- 0.07 g/worm) and higher survival (83%) in the commercial potting soil. The potting soil was amended with three forms of Zn (ZnCl<sub>2</sub>, ZnO bulk powder, ZnO NP) over a range of Zn concentrations to establish an EC<sub>10</sub> and EC<sub>50</sub> for *Eisenia fetida*. A split of the experimental soils was aged under constant temperature and moisture conditions for 28 days and the exposure experiment was repeated to assess the impact of ageing on the effect concentrations.

**MP033 Incorporating Invertebrate Community Data and Bioaccessibility Estimates into an Ecological Risk Assessment of a Shooting Range** S.R. Bowman, The Ohio State University / Evolution, Ecology, and Organismal Biology, The Ohio State University / Department of Evolution, Ecology, and Organismal Biology, The Ohio State University; J.L. Bryant, The Ohio State University / Entomology; R. Lanno, Ohio State University / Department of Evolution, Ecology, and Organismal Biology. Our study is an attempt to incorporate invertebrate community data and bioaccessibility into the site-specific ecological risk assessment of a shooting range. The study site is a private shooting range located in central Ohio. Areas of interest for this study include the main shotfall area that receives spent

ammunition from a trap and skeet range (2899 ± 1228 mg/kg soil Pb), an area where Pb pellets were excavated (Fall 2009; 628 ± 44 mg/kg soil Pb), and a reference area (1356 ± 86 mg/kg soil Pb) with less total Pb. Ground beetles were collected from 2008 to 2012 using pitfall traps in shotfall, reference, and lead extracted areas. Forty-five species were collected during the first two years. Overall species richness did not differ between sites; however, an indicator species analysis suggested that six species were indicators of the reference site, while one species was an indicator of the shotfall site. We hope that continued beetle and invertebrate sampling will contribute to our understanding of indicator species at the shooting range. We estimated Pb bioaccessibility to shrews (*Blarina brevicauda*) using a modified *in vitro* gastrointestinal method based on shrew digestive physiology. Field soils and earthworms were analyzed for bioaccessible Pb. Using this method, bioaccessible Pb was 5-10% of the total Pb in the tested soils. For earthworms exposed to field soils for 28 days, bioaccessible Pb ranged from < 1% - 4% of the total earthworm Pb. In the future, we hope to collect earthworms and shrews from the shooting range for the determination of Pb concentrations in their tissues. If a correlation is established between field Pb levels in shrews and the relative bioaccessibility, then these modified methods may be a useful tool for Pb exposure estimates for site-specific ecological risk assessment.

**MP034 Assessment of health risks associated with arsenic exposure from rice contamination near abandoned metalliferous mines** W. KIM, J. LEE, M. PAIK, A. KUNHIKRISHNAN, J. YOO, J. KIM, D. KIM, National Academy of Agricultural Science / Chemical Safety Division. To assess the ecotoxicological effects of arsenic (As) in agricultural environment and to give us a basic information for adequate management of As contaminated fields, As was analyzed in soils, irrigation water, and rice collected grown the paddy soils near abandoned metalliferous mines in Korea. The cancer risk probability (R) via rice intake grown near abandoned mines in each age-gender population exceeded the acceptable cancer risk of 10<sup>-6</sup> ~ 10<sup>-4</sup> for regulatory purpose. Particularly, cancer risk probability for children less than six years old was estimated to be high at 5.75 × 10<sup>-4</sup> compared to other age groups. Besides, hazard quotient (HQ) values for all age-gender categories did not exceed 1.0, implying that potential non-cancer toxic effect may not be caused by long-term exposure via As-enriched rice consumption. Human HaCaT cell viability, total antioxidants, and activities of antioxidant enzymes were also estimated on As contaminated polished rice grown adjacent to abandoned mines. The HaCaT cell viability in the As contaminated polished rice was inhibited in a time-dependent manner, ranging from 5-25%. Likewise, time-dependent total antioxidants were significantly decreased to 13-22 mM at 72 h post-incubation than in control. Enzyme activities of scavenging reactive oxygen species (ROS), were 2-5 folds induced compared to control. It demonstrates that mine-impacted polished rice induced HaCaT cell toxicity associated with oxidative stress.

**MP035 Nonlinear Estimation of Mineralization Half-Life by <sup>14</sup>CO<sub>2</sub> Production from MGA, Spectinomycin, Lincomycin, and Ceftiofur in Aerobically Incubated Soil** W. Smolenski, Pfizer Animal Health / Metabolism & Safety; R. Greening, Pfizer Animal Health; B. Hummel, Pfizer Animal Health; S. Lesman, Pfizer Animal Health; J. Robinson, Pfizer Animal Health / Veterinary Medicine Pharmaceuticals. Veterinary drug residues excreted by livestock typically enter soil systems. If the residue persists, the possibility of environmental effects (i.e. bioaccumulation, antibiotic resistance development, toxicity) may increase. Rapid degradation may reduce the likelihood of detrimental environmental effects. Environmental modeling applications typically use half-life (DT<sub>50</sub>) to predict environmental concentrations. If transformation data are not available, conservative DT<sub>50</sub> estimates based on mineralization to CO<sub>2</sub> may be used. Some countries regulatory authorities use the time to reach 50% mineralization (TR50%) as an estimate of DT<sub>50</sub>. When xenobiotics are transformed in soil, not all of the carbon in the molecule becomes CO<sub>2</sub>. Some carbon will bind to the soil or be incorporated into cellular components. Therefore, using the time to reach 50% mineralization is a poor estimate of DT<sub>50</sub>. To calculate half-life based on mineralization (M½), CO<sub>2</sub> production data were fit to a first-order or a sigmoidal logistic nonlinear model that estimated the maximum CO<sub>2</sub> produced at infinity and then determined the time at which mineralization would be at half of this maximum. In these studies each <sup>14</sup>C-radiolabeled drug was amended to 3-4 soils with different physical properties and incubated aerobically for up to 120 days. <sup>14</sup>CO<sub>2</sub> was collected in NaOH and scintillation counted. Soils were periodically extracted and analyzed



for parent and metabolites. All four drugs were transformed in soil and ultimately mineralized to CO<sub>2</sub>. The mean parent compound DT<sub>50</sub> in soil for ceftiofur, MGA and lincomycin were 0.04, 12.5 and 5.8 days and the mean mineralization M<sub>1/2</sub> values for CO<sub>2</sub> production were 13.4, 80.6 and 56.4 days, respectively. For the antibiotic spectinomycin, parent compound disappearance data were not available. The mean M<sub>1/2</sub> of spectinomycin in soil was 33 days based on CO<sub>2</sub> production. Predicting estimates of mineralization half-life from a model that estimates the maximum CO<sub>2</sub> produced is more appropriate than using the time to reach 50% mineralization, but is still conservative. Also, when obtaining an adequate amount of radiolabels drug is prohibitive or toxic at higher concentrations to soil bacteria, and parent compound loss cannot be followed because of analytical reasons, M<sub>1/2</sub> may be substituted as a conservative estimate of half-life.

**MP036 Acute toxicity analysis of urban septic tank sludge** P.B.

Miqueletto, R. Chagas, C. Veronesi, C. Soprani, S. Cassini, R. Franchi, University of Espírito Santo. The objective of this work was to evaluate toxic effects of sludge samples collected from urban septic tanks and upflow anaerobic sludge blanket (UASB) from a wastewater treatment plant located in the University of Espírito Santo. The method employed was the acute toxicity test Microtox based on the bioluminescence inhibition of the marine photobacterium *Vibrio fischeri*. It consists in a widespread methodology with many advantages such as ecological relevance, sensitivity, reproducibility, standardization and simple execution. A series of physical-chemical parameters were also analyzed based on Standard Methods for Examination of Water and Wastewater 20<sup>th</sup> (APHA – 1998). Sludge from four septic tanks was used in the study. From each tank four samples were collected, each aliquot representing a distinct depth, being classified as: 1. scum; 2. liquid waste, 3. superficial sludge; 4. bottom sludge and 5. mixture from all the previous. From the UASB equivalent fractions were also sampled. The tests were executed according to the Microtox User's Guide Manual and results were expressed in EC<sub>50</sub> (Effective Concentration), that represents the sample concentration in which 50% of the bacterial luminescence is inhibited. Only in one of the septic tanks, located in a public building, toxic effects could be verified in the upper layers "scum" and "liquid waste". All samples from the bottom fractions presented higher toxic effects (EC<sub>50</sub>= 9.4%) when compared to the fractions above. The average EC<sub>50</sub> for fraction 3 was 12.6% and 17% for the mixture, meaning even lower toxicity, possibly due to the influence of upper fractions that are stored in great volumes inside the tanks. The sludge from UASB presented higher toxic effects on top side (EC<sub>50</sub>= 5.5%) in comparison to the bottom (EC<sub>50</sub>= 8%), but lower toxicity when compared to tanks samples, since it was analyzed in a dilution of 50 parts in 200, while septic tank samples were diluted in one part in 200. All samples presented elevated levels of TKN, P and COD and were considered as stabilized sludge samples, since the relation VS/TS (volatile solids/total solids) was below 65%. Although many studies have applied the *Vibrio fischeri* test to a range of environmental samples, the analysis of sludge from septic tank represents a new approach, essential to evaluate the risk of releasing this residue in the environment or even ensure the quality required for its application in other activities.

**MP037 Correlating Environmental and Biological Monitoring using GCMS: Commuters' Exposure to Volatile Organic Compounds in the Greater Houston Area** S.L. Tarver, Texas Southern University / Environmental Science and Technology.

The general population is exposed to a large number of pollutants continuously emitted into the environment. Urban air pollution caused by automobile and industrial sources can aggravate many respiratory conditions, and lead to additional acute and chronic health effects. Exposure to volatile organic compounds (VOCs), specifically benzene, toluene, ethylbenzene, and the isomers of xylene (BTEX) is ubiquitous since two common sources are vehicular traffic and tobacco smoke. Bio-monitoring of human specimens is one approach to evaluating the toxicological effects of the compounds and their risks to human health. In past studies, the analysis of non-occupational exposure of VOCs has been conducted using blood specimens, or exhaled breath also referred to as "expired air". Few studies have undertaken urine analysis as a viable option for evaluation of non-occupational exposure to VOCs. The evaluation of environmental exposures to VOCs has been conducted by means of solvent extraction and headspace techniques. Solid-phase microextraction (SPME) has proved to be an excellent method for the analysis of semi-volatile and most volatile organic compounds, but purge and trap (PT) remains the technique of choice for the sample prep and analysis for VOCs. The proposed

plan is to examine the viability of purge and trap gas chromatographic (GC) determination of VOCs in urine with mass spectrometric (MS) detection. The aim of this study is to provide data that supports the use of urine analysis as a biomarker in non-occupational VOC exposure. Air quality inside the vehicles of volunteers without occupational exposure will be monitored for 1-hr and 24-hour sampling periods. Urine samples will be collected from the volunteers and analyzed for detectable concentrations of the VOCs to assess human exposure to them.

**MP038 Development of Dried Blood Spot Reference Material** J.L. Rein-

et al. / Environmental Health Sciences, NIST / Analytical Chemistry Division; S. Long, C. Bryan, M. Schantz, NIST. The practice of collecting human blood on filter paper and using it for population screening dates back to the early 1960s when an assay was developed to measure phenylalanine in dried blood spots (DBS). Though this innovated approach was developed for determination of metabolic diseases, some studies now report the use of DBS for the quantification of organic contaminants and elements. Two NIST Standard Reference Material (SRM) 1958 Organic Contaminants in Fortified Human Serum and SRM 955c Caprine Blood, Level 4 were applied to DBS cards. Initially, measurements of perfluorooctane sulfonate (PFOS) and mercury (Hg) were made on the DBS. Preliminary results show that PFOS and Hg are measurable and the reproducibility of the measurements is good. Future work will include the measurement of additional organic and inorganic compounds. Three other marker organic compounds have been chosen to analyze in the blood spot material. 4,4'-DDE was chosen as a marker compound for organochlorine pesticides. The polychlorinated biphenyl (PCB) congener 153 was chosen as a marker compound for PCBs. Polybrominated diphenyl ether (PBDE) 47 will be used as a marker compound for PBDE exposure. Measurements of arsenic, iodine, and lead in the DBS are planned. With improvements in analytical instrument sensitivity, there is expected to be a surge in the use of DBS as a resource for chemical measurements. In anticipation of the surge, we aim to develop a reference material that will help to determine the recoverability and stability of different analytes from blood spots.

**MP039 Evaluating Dietary Consumption Advisories in Reducing Human Exposure to Persistent Organic Pollutants** M. Binnington,

University of Toronto Scarborough / Dept. of Physical & Enviro. Science, UTSC – Dept. Physical & Environmental Sciences; C. Quinn, F. Wania, University of Toronto Scarborough / Physical & Environmental Sciences. Human exposure to persistent organic pollutants, including polychlorinated biphenyls (PCBs), mainly occurs through dietary intake of contaminated food. Thus, regulatory bodies issue dietary consumption advisories to describe safe intake levels for food items of concern, particularly piscivorous fish. Our study goal was to estimate the effectiveness of published fish advisories in reducing contaminant exposure among susceptible subpopulations, namely children and women of childbearing age. We used the time-variant mechanistic model CoZMoMAN to calculate human bioaccumulation of PCBs under different scenarios of guideline adherence for both hypothetical constant and realistic time-variant chemical emissions. Specifically, we have compared prenatal, postnatal, and childhood contaminant body burdens under different fish consumption advisory scenarios. We also calculated exposure reduction potential for a range of theoretical chemicals varying in phase partitioning and degradability to identify for which chemicals guidelines are most/least effective. We found the most significant predictors of advisory effectiveness to be degree of compliance with specified advisories, and the rate of human contaminant metabolism. More specifically, our model suggests eliminating or reducing fish consumption only during the 1.5-year period of pregnancy and breastfeeding is ineffective in reducing prenatal and postnatal exposures of offspring compared to maternal default fish consumption. Also, advisories proved most effective in reducing measured exposures during periods of increasing, as opposed to decreasing, contaminant emissions. In summary, dietary advisory effectiveness depends mainly on length of compliance period, *in vivo* contaminant half-life, and time from peak emissions. Greatest success in exposure reduction is achieved during long-term dietary adjustment for chemicals readily degraded by humans, during increasing chemical emissions. This clarification of variables critical in predicting guideline success may lead to improved advisories for persistent organics in the future.

**MP040 F2-isoprostanes Determination by Solid Phase Extraction and Liquid Chromatography with Tandem Mass Spectrometry in Human Blood Samples** W. Guo, S.E. Petropoulou, California Department of Toxic Substances Control; N. Holland, School of Public Health, University of California at Berkeley; M. Petreas, California Department of Toxic Substances Control, DTSC; J. Park, California Environmental Protection Agency / Department of Toxic Substances Control, California Environmental Protection Agency / Department of Toxic Substances Control, Environmental Chemistry Laboratory, California Environmental Protection Agency / Research Scientist. Isoprostanes (iPs) are prostaglandin like products formed in vivo by free radical-catalyzed nonenzymatic peroxidation of arachidonic acid. Several F2-iPs are established sensitive and reliable protein markers for oxidative stress. However, it's still a challenge to measure F2-iPs in human blood due to their relatively low quantities. In the past, GC-MS/MS and immunological approaches were used for isoprostane analysis. Although they are sensitive methods, they have complicated sample preparation involving derivatization steps in GC method and lack of specificity in immunoassay. We present a novel LC-MS/MS method using isotope dilution to simultaneously determine six F2-iPs isomers (8-iso-PGF2 $\alpha$ , 8-iso-15(R)-PGF2 $\alpha$ , 15(R)-PGF2 $\alpha$ , PGF2 $\alpha$ , 11 $\beta$ -PGF2 $\alpha$ , 5-iPF2 $\alpha$ -VI) in a single human blood sample (500 $\mu$ L). F2-iPs were detected using an AB Sciex 5500 QTrap mass spectrometer, coupled with a Shimadzu 20AC LC system. Ionization was achieved using electrospray in the negative ESI mode. Each analyte was optimized in several solvent combinations in order to achieve maximum ionization. The most abundant daughter ion was used for quantitation and the secondary ion was used as a confirmation ion. For the separation of the F2-iPs, a base deactivated silica C8 column was used and the total run time was 27 min. We also tested different SPE sorbents to minimize matrix-related suppression effects. Validation of this most robust and accurate SPE protocol is currently underway. To our knowledge, this is the first method using LC-MS/MS for the determination of six F2-iPs in human serum samples in one injection. The finalized method will be incorporated in large biomonitoring and molecular epidemiology studies to measure oxidative stress induced by chemical exposures, or other factors. The views expressed herein are those of the authors and do not necessarily reflect those of the California Department of Toxic Substances Control.

**MP041 Measurement of Hydroxy and Parent-PAHs in Urine and Atmospheric Particulate Matter** O. Motorykin, Oregon State University / Department of Chemistry; S. Simonich, Oregon State University / Depts. of Chemistry and Environmental & Molecular Toxicology; J.E. Schlau, Oregon State University / Chemistry. Humans are exposed to Polycyclic Aromatic Hydrocarbons (PAHs) through polluted air and diet. Broiled, smoked or grilled food contain higher amounts of PAHs compared to uncooked or boiled food. In air, PAHs are emitted from incomplete combustion of fuels and are adsorbed on fine particulate matter, which can be deposited in lungs, causing adverse health effects and cancer. When in the human body, parent-PAHs are metabolized to hydroxy-PAHs and excreted via urine. By comparing the parent PAH composition of particulate matter (PM) and the hydroxy-PAH composition of urine, the metabolic pathways of PAHs can be studied. An analytical method was developed to simultaneously measure 33 hydroxy-PAHs and 19 parent PAHs in urine and particulate matter using gas chromatography/mass spectrometry (GC/MS). Urine samples were deglucuronized with  $\beta$ -glucuronidase/aryl sulfotase and solid phase extracted (SPE) with the combination of C18 and Plexa stationary phases. PM was extracted from filters with the use of Accelerated Solvent Extraction (ASE). The urine extracts and aliquots of PM extracts were mixed with an internal standard and derivitized with *N*-methyl-*N*-tert-butyldimethylsilyl-trifluoroacetamide (MTBSTFA). The derivitized samples were analyzed in electron impact ionization mode with GC/MS. Stable isotope labeled surrogates were used to account for loss throughout the analytical method. Recovery experiments were conducted using a combination of C18 and Plexa SPE columns and elution with a 1:1 mixture of dichloromethane:ethylacetate. Most of the recoveries from urine ranged from 80 to 120%. PM recovery showed that dichloromethane should be used to extract the analytes when using ASE. Intra- and inter-day variability of the measurements and stability of the derivitized product were also investigated. Results showed that the 33 OH-PAH formed stable products over a two week storage period. The stability of OH-PAHs in frozen urine was also studied. Results showed that many OH-PAH were stable in frozen urine for a one week period. However, the dihydroxynaphthalenes were not stable in frozen urine for a one week period.

**MP042 Occurrence and Profiles of Phthalates in Foodstuffs from China and their Implications for Human Exposure** Y. GUO, Wadsworth Center, New York State Department of Health; Z. Zhang, L. Liu, Y. Li, N. Ren, International Joint Research Center for Persistent Toxic Substances (IJRC-PTS), State Key Laboratory of Urban Water Resource and Environment, Harbin Institute of Technology; K. Kannan, Wadsworth Center, New York State Department of Health. Phthalate esters are used in a wide variety of consumer products, and human exposure to this class of compounds is widespread. Nevertheless, studies on dietary exposure of humans to phthalates are limited. In this study, nine phthalate esters were analyzed in eight categories of foodstuffs ( $n = 78$ ) collected from Harbin and Shanghai, China, in 2011. Dimethyl phthalate (DMP), diethyl phthalate (DEP), dibutyl phthalate (DBP), di-*iso*-butyl phthalate (DIBP), butyl benzyl phthalate (BzBP), and diethylhexyl phthalate (DEHP) were frequently detected in food samples. DEHP was the major compound found in most of the food samples, with concentrations that ranged from below the limit of quantification (LOQ) to 762 ng/g wet weight (wt). The concentrations of phthalates in food samples from China were comparable to concentrations reported for several other countries, but the profiles were different; DMP was found more frequently in Chinese foods than in foods from other countries. The estimated daily dietary intake of phthalates (EDIDie) was calculated based on the concentrations measured and the daily ingestion rates of food items. The EDIDie values for DMP, DEP, DIBP, DBP, BzBP, and DEHP (based on mean concentrations) were 166, 70.7, 906, 1100, 481, and 2440 ng/kg-bw/d, respectively, for Chinese adults. The EDIDie values calculated for phthalates were below the reference doses suggested by the United States Environmental Protection Agency (EPA). Comparison of total daily intakes, reported previously based on an urinary biomonitoring study in China, with the current dietary intake estimates suggests that diet is the main source of DEHP exposure in China. Nevertheless, diet accounted for only < 10% of the total exposure to DMP, DEP, DBP, and DIBP, which suggested the existence of other sources of exposure to these phthalates.

**MP043 Optimization of speciation conditions for Arsenic in human urine using isocratic HPLC-ICP-MS** I. Sen, W. Zou, R. Gajek, California Department of Public Health; J. She, Environmental Health Lab / CA Department of Public Health. Arsenic is toxic and carcinogenic in humans depending on the chemical nature and oxidation states of the compounds. In order to determine the extent of toxic exposures to human and to identify the contribution of inorganic and seafood related Arsenic exposure, it is very important to ascertain the measurements of total Arsenic along with the quantification of each different species of Arsenic present in human urine. In the body, Arsenic is metabolized to several different inorganic and organic species, including trivalent Arsenic (As-III) and pentavalent Arsenic (As-V), and the methylated species such as Monomethylarsonic acid (MMA), and Dimethylarsinic acid (DMA), in addition to Arsenobetaine (AsB) and Arsenocholine (AsC) which stays unaffected in the body. Conventionally HPLC gradient pump is used in combination of different mobile phase composition to optimize the baseline separation of each Arsenic species as well as reduce the total elution time. In this study we used HPLC isocratic pump with single mobile phase composition and pH conditions to optimize the baseline separation of each Arsenic species with the shortest possible elution time. The separation was performed with Hamilton PRP-X100 $\text{\textcircled{R}}$  anion exchange column using Agilent 1200 HPLC isocratic pump along with Agilent 7700 inductively coupled plasma mass spectrometry as a detector. The results showed that each of the six Arsenic species was well separated and LOD of each of the species is approximately 1 ppb. The baseline resolved separation of Arsenobetaine (AsB) with As-III is critical since in real urine sample AsB could present in 100 ppb range with very low As-III (1-2 ppb) concentration. Besides, As-III tends to oxidize at room temperature to As-V during the course of long analyses. In order to prevent the oxidation of As-III, we introduced several steps including i) a pH 6.0 sample preparation buffer ii) removing oxygen present in the mobile phase by degassing with Argon and iii) use of low temperature (-70 $^{\circ}$ C) for long term storage. The above-mentioned method can be applied to analyze Arsenic species in human urine.

**MP044 PCBs and OH-PCBs in Mothers and Their Children Living in Urban and Rural Communities** R.F. Marek, University of Iowa / Civil & Environmental Engineering, The University of Iowa / College of Engineering; K. Wang, P.S. Thorne, J. DeWall, The University of Iowa / College of Public Health; K.C. Hornbuckle, University of Iowa / Department. of Civil

& Environmental Engineering, IIHR-Hydrosience and Engineering. We have analyzed human blood serum collected since 2008 from adolescent children and their mothers in the urban East Chicago, Indiana area ( $n > 100$ ) and the rural Columbus Junction, Iowa area ( $n > 100$ ) for all 209 PCB congeners and 11 hydroxylated PCBs (OH-PCBs). East Chicago is a heavily-industrialized residential community on the southwestern shore of Lake Michigan. Bisecting the area, and passing within 2 km of two schools, is the Indiana Harbor and Ship Canal from which at least 7 kg PCBs per year volatilize. In contrast, residents of the rural Columbus Junction area have no known PCB exposure from current or past industrial sources. We used highly selective methods to analyze samples for all 209 PCBs as 160 chromatographically-resolved peaks and 11 hydroxylated PCBs. A full suite of quality control measures, including standard reference materials, was applied to optimize and assess the analytical method for our target compounds. Analysis of the first year of serum collection suggests that East Chicago children are enriched in lower molecular weight, more volatile PCBs. There is no statistically significant difference between East Chicago and Columbus Junction sum PCBs or sum OH-PCBs. Interestingly, children have about half the amount of PCBs but about the same amount of OH-PCBs as their mothers. Though sum OH-PCBs correlate with sum PCBs, analysis of individual metabolites and their likely parent congeners offer no clear metabolism pathway preference. Following analysis of a second round of blood sampling one year later, these trends are examined for continuity within the second year of data.

**MP045 Phthalates and Brominated Flame Retardants: Indoor Concentrations, Dermal Wipes and Urine Biomonitoring** S.R. Chaudhuri, University of Toronto / Department of Chemical Engineering and Applied Chemistry, University of Toronto; A. Saini, E. Goosey, University of Toronto; H. Qi, Harbin Institute of Technology; M. Venier, Indiana University / School of Public and Environmental Affairs, Indiana University; M.L. Diamond, University of Toronto / Department of Geography and program in planning, University of Toronto / Department of Geography. As part of a detailed indoor and exposure assessment of 5 individuals and their homes, we measured concentrations of phthalate esters and brominated flame retardants in indoor air, dust, and window films in several rooms per homes, and dermal wipes and urine (the latter only for phthalate metabolites) per individual. Here we report on the relationship among several of the indoor levels and those in dermal wipes. Three consecutive wipes were taken on four consecutive days for each of the five individuals. The wipes were of the left hand, the right hand and forehead. Standard laboratory procedures and QA/QC were followed for sample extractions, clean up and analysis by GC-MS. For phthalates, higher molecular weight di(2-ethylhexyl) phthalate (DEHP) and diisononyl phthalate (DiNP) concentrations were 1 to 2 orders of magnitude higher in indoor dust, window films, and dermal wipes compared to lower molecular weight phthalates. Levels of DEHP and DiNP in dust also varied by an order of magnitude between individual homes. DEHP and DiNP concentrations in hand wipes were greater than those of forehead wipes. Metabolites of diethyl phthalate (DEP) and DEHP were found to be the highest in participant urine samples. For brominated flame retardants (BFRs), concentrations in indoor dust samples were highest for ethylhexyl-tetrabromobenzene (TBB), bis(2-ethylhexyl) tetrabromophthalate (TBPH), and tris-dichlorophenyl phosphate (TDCPP). DecaBDE replacements brominated trimethylphenylindane (OBIND) and decabromodiphenylethane (DBDPE) were also present but at lower concentrations and frequency of detection. The concentrations of BFRs in dermal wipes followed these patterns.

**MP046 QuEChERS sample preparation followed by UPLC-MS/MS analysis of DOSS in egg and blood tissue collected from birds impacted by Deepwater Horizon** A. Yeudakimau, University of Connecticut / Center of Environmental Sciences and Engineering; A. Provas, University of Connecticut / Center for Environmental Sciences and Engineering; C.R. Perkins, University of Connecticut / Center for Environmental Sciences and Engineering, University of Connecticut / Center for Environmental Sciences and Engineering; J. Stuart, University of Connecticut / Center for Environmental Sciences and Engineering. After the devastating Deepwater Horizon offshore rig explosion in the Gulf of Mexico on April 20<sup>th</sup> 2010, a series of measures were undertaken to minimize the potential for crude oil to spread and impact coastal areas and their associated wildlife. Oil dispersant known as Corexit 9500 was widely used throughout the affected region. Primary components of Corexit 9500 are sorbitan, butanedioic acid, propylene

glycol, and Dioctyl Sulfosuccinate Sodium Salt (DOSS). A quantification method was developed for the determination of DOSS in various types of bird egg and blood tissues. Sample preparation utilized a QuEChERS (Quick, Efficient, Cheap, Effective, Rugged and Safe) extraction technique. A Waters Acquity UPLC system equipped with a tandem mass spectrometer was used for developing a separation method and achieving significantly lower detection limits when compared to alternative methods, including traditional high pressure liquid chromatography. The method provided an excellent selectivity and sensitivity for DOSS with a detection limit as low as 500 pg/mL and with associated analyte recoveries in the range of 90-115%. The extraction efficiency was monitored by adding a surrogate compound, sodium octyl sulfate-d17 (SOS-d17), to every sample with typical recoveries of 60-95%. In summary, this method can provide a rapid and easy preparation and sample analysis for the determination of DOSS in various tissue matrices.

**MP047 Speciation of iodide and iodate using gradient IC-ICP-MS** W. Zou, California Department of Public Health; K. Ting, J. Quinn, California Department of Toxic Substances Control; J. She, California Department of Public Health. MeI is effective in controlling a broad spectrum of soil borne pathogens and has the potential to replace MeBr in soil fumigation. However, MeI is carcinogenic and exhibits moderate to high acute toxicity for inhalation and ingestion. Therefore, the environmental fate of MeI is of great concern. In aqueous environment, MeI either degrades to iodide or to iodate. With oxidation states ranging from -1 to +7, the predominant states of iodine in aqueous systems are -1 (iodide,  $I^-$ ) and +5 (iodate,  $IO_3^-$ ). In reducing environments, aqueous iodine usually occurs as the mobile monovalent anion,  $I^-$ . Under more oxidizing conditions, iodine is present as  $IO_3^-$ . Therefore, it is critical to simultaneously monitor MeI,  $I^-$ , and  $IO_3^-$  levels in aqueous solution. In the present study, a modified Perkin-Elmer IC-ICP-MS system (Series 200 LC pumps and Elan 6100 ICP-MS, plus a Dionex suppressor controller RFC-10) was applied to analyze iodine species. The column was Ionpac AS16 (4x250mm) with AG16 (4x50mm). At a flow rate of 1 mL/min, a gradient of  $H_2O$  and 350 mM NaOH at 0%B-100B% in 15 min was good enough to separate iodate and iodide. A RFC-10 controlled ASRS 300 suppressor worked in external water mode with a voltage of 300 mA. ICP-MS was optimized based on the criteria of highest sensitivity of iodine and rhodium (internal standard), ratio of cerium oxide to cerium less than 3%, ratio of doubly charged barium to barium ions less than 5%. Data of iodine element was acquired at  $m/z$  127 with dwell time of 250 ms in dual detector mode. The results showed that iodate and iodide were well separated and LOD of both species could reach 1 ppb. The above-mentioned method can be easily applied to analyze iodine species in human blood and urine.

**MP048 Studying matrix effects on perchlorate assay in human urine using IC coupled with conductivity detector and ESI-MS/MS** X. Wang, California Department of Public Health; W. Zou, R. Gajek, J. She, California Department of Public Health. Perchlorate interferes with iodine uptake into the thyroid gland and is considered as a contaminant by US EPA, ascertaining the necessity of monitoring perchlorate in human body fluids. The state of the art perchlorate analysis in urine is ion chromatography (IC) coupled with conductivity detector and electrospray ionization (ESI) triple-quadrupole mass spectrometry (MS/MS). Conductivity detector works well with simple sample matrix, but has high backgrounds and broad tailing peaks when large amount of anions like sulfate, chloride, and carbonate are present. ESI-MS/MS improves the sensitivity and specificity compared with conductivity detector, but substances in urine matrix may co-elute with perchlorate and cause ion suppression or ion enhancement in ESI. In order to reduce matrix effects, urine samples are typically diluted before analysis. Although dilution is effective in most cases, it affects the method minimal detection limit (MDL) and minimal reporting limit (MRL). In order to improve MDL and MRL, sample pretreatment and cleanup methods have been investigated by other labs using SPE or cation-exchange resin, at the expense of having more complex steps and lower recovery rate. The purpose of our study was to compare matrix effects using conductivity and ESI-MS/MS detectors, and to find the best approach to compensate or subdue matrix effects in urine perchlorate assay. In our preliminary study, perchlorate was eluted at 11 min using a AS16 column (2x250mm) along with a AG16 guard column (2x50mm) and data was acquired using conductivity detector. We compared results among water samples of different sources (tap water, Milli Q water, HPLC grade water, and LC-MS grade water) spiked with



1 ppm of perchlorate. Interestingly, results show that Milli Q water had lowest background anion peaks. Retention time shift, peak shape, MDL, MRL, calibration curve linear range, accuracy, and precision will be studied in in-house made synthetic urine, anonymously collected in-house pooled urine, and synthetic urine from different vendors at different dilution factors spiked at different perchlorate levels. If necessary, we will investigate different sample cleanup approaches to improve MDL and MRL.

**MP049 Urinary Concentrations of Six Parabens in the Alberta Population** D.A. Birkholz, ALS Laboratory Group / Research and Toxicology, ALS Laboratory Group / North American Centre of Excellence; C. Sandau, Chemistry Matters; S. Genuis, University of Alberta; B. Elmayergi, B. O'Neill, M. Ralitsch, ALS Laboratory Group. Parabens are esters of p-hydroxybenzoic acid which are used as antimicrobial preservatives, in cosmetics, pharmaceuticals, and food and beverage processing. The estrogenic activity of parabens in animals and the presence of these compounds in human breast tissue and urine have raised concern on their impact on human health. An in-line solid phase extraction (SPE) method followed by liquid chromatography-tandem mass spectrometry was validated for the determination of parabens in human urine. One of our main findings on the use of protein precipitation methods greatly impact reported paraben concentrations in urine. Fifty-nine samples of urine were received from two medical researchers. Geometric means were determined and compared with the appropriate National Health and Nutrition Examination Survey (NHANES) data. Geometric means were determined for methyl paraben (MP), ethyl paraben (EP), propyl paraben (PP), butyl paraben (BP), isobutyl paraben (IBP) and benzyl paraben (BzP). For the entire population set aged 12 – 67 geometric means were 24.0 (MP), 13.2 (EP), 6.9 (PP), 0.72 (BP), 0.70 (IBP) and 0.36 (BzP) ng/mL. These values are generally lower than those reported by NHANES for appropriate ethnic group comparison from the 2009-2011 data set for MP, EP, PP and BP (n = 826). The observed range of values in our study are as follows: 0.74 – 666 (MP), 3.9 – 247 (EP), 1.2 – 496 (PP), 0.35 – 9.7 (BP), 0.35 – 9.8 (IBP), and 0.35 – 0.69 (BzP). Geometric means observed for females (n = 41) aged 20 – 67 were: 26.7 (MP), 13.8 (EP), 7.1 (PP), 0.81 (BP), 0.80 (IBP) and 0.35 (BzP). These are generally lower than those reported by NHANES. The ranges for females was 0.74 – 666 (MP), 4.5 – 247 (EP), 1.2 – 331 (PP), 0.35 – 9.7 (BP), 0.35 – 9.8 (IBP) and 0.35 – 0.37 (BzP). A great deal of interest has arisen in Canada concerning the human body burdens of parabens, especially in women of child bearing age and in pregnant women. As a result, Health Canada, Statistics Canada and our laboratory will conduct a much larger study. Furthermore, our research team is very interested in looking at circulating blood levels to determine whether medical intervention is required to eliminate and/or reduce human body burdens of environmentally relevant chemicals.

**MP050 Comparison of IC-MS, HILIC LC-MS, and RP LC-MS of Perchlorate Analysis in Human Urine** W. Zou, X. Wang, R. Gajek, J. She, California Department of Public Health. Perchlorates are often produced by natural processes, but can also be produced artificially as a component of solid rocket fuel, or used on spacecraft and submarines as emergency oxygen supply. Unfortunately, perchlorate interferes with iodine uptake into the thyroid gland. In January 2008, California's Department of Toxic Substances Control stated that perchlorate is a threat to human health and water resources. In February, 2011, US Environmental Protection Agency (EPA) stated that perchlorate is a contaminant. Therefore, monitoring perchlorate in human body fluids is critical to protect public from potential adverse effects. In the present study, with IC-MS, HILIC LC-MS, and RP LC-MS, we wanted to see which platform was better for perchlorate analysis in human urine. A triple quadrupole MS/MS mass spectrometer (API-5000, Sciex) was coupled either with an IC system (ICS-2000, Dionex) or an LC system (LC-20, Shimadzu). On the IC, an anion exchange column (AS-20, 250 x 2 mm, Dionex) protected by a guard column (AG-20, 50 x 2 mm, Dionex) was used together with an ion suppressor (ASRS-300, Dionex). On the LC, either an HILIC column (Excel 2 CN, 150 x 2.1 mm, ACE) or a modified RP column (Excel 2 C18-PFP, 150 x 2.1 mm, ACE) with suitable guard column was used. Unlabeled perchlorate ( $\text{HClO}_4$ ) was monitored in negative ESI mode using MRM transition of m/z 99 → m/z 83, m/z 101 → m/z 85, and labeled internal standard ( $\text{HClO}_4^{18}$ ) was acquired using m/z 107 → m/z 89. Compound specific and source parameters were optimized to maximize the sensitivity of the assay. Initial Demonstration of Capability (IDC) for each assay will be conducted immediately after the optimization

process. The method with highest sensitivity and lowest matrix effects will be chosen for routine analysis of perchlorate in human urine.

**MP051 Genotoxic and cytotoxic damage produced by mercury on blood cells of the common carp (*Cyprinus carpio*)** M. Galar-Martinez, National School of Biological Sciences, IPN / Laboratory of Aquatic Toxicology, Department of Pharmacy; J. Nunez-Betancourt, National School of Biological Sciences, IPN; S. Garcia-Medina, National School of Biological Sciences, IPN. / Laboratory of Aquatic Toxicology, Department of Pharmacy; L. Gomez-Oliván, School of Chemistry, Autonomous University of Mexico State.. Mercury is one of the most important heavy metals in the aquatic ecosystems, since it is not removed by natural processes, can be incorporated into the food chain due to bioaccumulation processes and can produce serious toxic effects on diverse aquatic species. In fish, for example, has been shown that this metal produces disorders in sensorial, food, reproductive and swimming behavior. However, regarding the genotoxic and cytotoxic damage, there is little information and such studies are essential to identify the mechanisms by which it operates. The aim of this study was to evaluate the cytotoxic and genotoxic effects of mercury in blood of common carp (*Cyprinus carpio*). In order to reach this aim, test organisms were exposed to different sublethal concentrations of the metal (0.001 mg / L and 0.01 mg / L) for 12, 24, 48, 72 and 96 h. After the exposure time, blood samples were obtained and the following biomarkers were evaluated: DNA damage using the micronucleus assay and apoptosis by the technique of in situ labeling of fragmented DNA (TUNEL). The results showed that mercury produced a marked induction of micronuclei frequency and apoptosis of blood cells in both test concentrations at all exposure times, in both cases peaking at 48 h, then decline, although always above the control. These results demonstrate the genotoxicity and cytotoxicity caused by mercury and the need for further toxicological studies of this metal.

**MP052 Short-term molecular-level effects of silver nanoparticle exposure on the earthworm, *Eisenia fetida*** O.V. Tsyusko, University of Kentucky / Department of Plant & Soil Sciences; S. Hardas, University of Kentucky / Department of Chemistry; W.A. Shoults-Wilson, Roosevelt University / Department of Biological, Chemical, and Physical Sciences; C. Starnes, University of Kentucky / Biostatistics, Epidemiology, and Research Design; G. Joice, University of Kentucky / Plant and Soil Sciences; D.A. Butterfield, University of Kentucky / Department of Chemistry; J. Unrine, University of Kentucky / Plant and Soil Sciences. Short-term changes in levels of expression of nine stress response genes and oxidative damage of proteins were examined in earthworms, *Eisenia fetida*, exposed to polyvinylpyrrolidone (PVP) coated silver nanoparticles (Ag-NP) and  $\text{AgNO}_3$  in natural soils. The worms were exposed at nominal Ag concentration of 100 and 500 mg  $\text{kg}^{-1}$  for 0, 1, 3, and 7 days. The responses varied significantly among days with seven out of nine analyzed genes having day as the most significant variable determining changes in gene expression. Among the days, the third day showed the highest number of significant changes in levels of gene expression and protein carbonyls relative to controls. Gene expression patterns for Ag-NPs and  $\text{AgNO}_3$  were similar, suggesting that ions are primarily responsible for the observed responses. Significant correlations of decreased gene expression with increased Ag soil concentration were observed for CAT and HSP70 on day three and for MT on day seven suggesting that genetic responses of these genes are determined by Ag soil concentration independent of the NP size and from whether the worms were exposed to ions or particles. Significant increases in the levels of protein carbonyls on day three were observed for all treatments indicating that *E. fetida* experienced oxidative stress three days after exposure to both particles and ions. Decreased expression and activity of CAT, one of the enzymes responsible for detoxification of highly reactive oxygen species  $\text{H}_2\text{O}_2$ , was also observed on day three. Decreases in CAT may lead to accumulation of  $\text{H}_2\text{O}_2$ , consistent with the increased protein carbonyls on day three. The results from this study suggest that Ag ions are primarily responsible for Ag NP toxicity in *E. fetida*. However, given that < 15% of Ag in the NPs was oxidized in these soils, dissolution of Ag-NPs is likely to occur after or during their uptake.

**MP053 Effect of Cd (II) and Pb (II) on Vetiver gene expression of a putative Voltage-Dependent  $\text{Ca}^{2+}$  Channel, TPC1** M. Rodriguez Hernandez, Universidad Autonoma de San Luis Potosi / Faculty of Chemistry; I. Bonifaz Arredondo, Instituto Potosino de Investigación Científica y Tecnológica A.C.; M. Alfaro De la Torre, J. Flores Flores, Universidad

Autonoma de San Luis Potosi / Faculty of Chemistry. There is a considerable interest in the development of low-cost, effective, affordable and environmentally friendly solutions to remediate sites contaminated with heavy metals. Phytoremediation is an interesting technology for this purpose. From the biotechnological point of view, the ability to remove metals by the plants can be improved by inducing their tolerance to the pollutants. Thus, the strategies followed by the plants for the metals uptake, the translocation and compartmentalization, need to be understood. The plant *Vetiveria zizanioides* has been used for soil and water remediation and it is a potential candidate species for the absorption of a wide range of heavy metals from metal-contaminated soils and water. However, the uptake mechanisms that this plant used to accumulate metals are still not very well known. This work is focused in the removal of divalent metals (Cd and Pb) by studying the transport mechanisms of those metals in *V. zizanioides*. We analyzed the uptake mechanism of Cd and Pb by the plant root through the transport mechanism of  $\text{Ca}^{2+}$ . Young plants ( $n=3$  per treatment) of equal size were grown hydroponically and amended with 0 – 15  $\text{mgL}^{-1}$  of each heavy metal individually for 10 days. The grown index as well as total chlorophyll of plants exposed to the heavy metals was not significant to controls. Moreover, a  $\text{Ca}^{2+}$ -permeable channel gene *VzTPC1* encoding a putative membrane protein was cloned from *V. zizanioides*. Our investigation revealed that the overexpression of *VzTPC1* induced by Cd in the nutrient solution was similar to the plants growing in a solution with calcium; whilst the gene expression of *VzTPC1* in plants exposed to Pb was only slightly correlated with the concentrations of the ion solution.

**MP054 Sex-specific gene expression in early life stage fathead minnows (*Pimephales promelas*) throughout development and after exposure to synthetic hormones** J.K. Leet, Purdue University / Forestry and Natural Resources; J.J. Amberg, United States Geological Sciences / Upper Midwest Environmental Sciences Center; A.W. Olmstead, Bayer CropScience / Mid-Continent Ecology Division, Bayer CropScience / Environmental Toxicology and Risk Assessment; G.T. Ankley, US EPA / Office of Research and Development, National Health and Environmental Effects Research Laboratory, Mid-Continent Ecology Division, U.S. EPA / National Health and Environmental Effects Research Laboratory; L.S. Lee, Purdue University / Dept. of Agronomy Crop, Soil & Environmental Sciences, Purdue University / Dept. of Soil, Crop & Environmental Sciences; M.S. Sepulveda, Purdue University / Forestry & Natural Resources and School of Civil Engineering, Purdue University / Forestry & Natural Resources &. There is evidence that exposure to endocrine disrupting chemicals (EDCs) during early life stages can alter sex differentiation in fishes. Fathead minnows (*Pimephales promelas*) are commonly used as a model fish species in endocrine disruption studies. However, limited knowledge exists on molecular pathways associated with sex differentiation in early life stages of this species. Changes in the expression of genes important in sex differentiation could be useful in evaluating the effects of EDCs on gonadal development and sex differentiation. Until this point no study has characterized the sex-specific baseline expression of genes in developing fathead minnows. Using a sex-linked DNA marker to verify gender, we evaluated the expression of genes involved in gonad development and sex differentiation (*dmrt1*, *cyp19a*, *cyp17*, *star*, *esr1*, *ar*) in developing fathead minnows (10 – 45 days post hatch; dph) to establish this baseline data. Expression of all genes remained relatively constant in males except for *cyp17*, which increased to almost 20 fold by 45 dph. Gene expression patterns in females varied more than those in the males, but generally expression increased 10 to 20 fold by 45 dph. Since these genes have been linked with sex differentiation, we hypothesize expression of these genes will be altered in a sex-specific manner when fathead minnows are exposed to androgens, estrogens, or mixtures of EDCs during this same life-stage. Preliminary results show a significant increase in *cyp19a* expression in males exposed to 1.5 ng/L 17 $\alpha$ -ethinylestradiol (a potent synthetic estrogen) between 10 and 20 dph, but no significant increase in expression was observed in females. Evaluation of these molecular markers in combination with gender identification is being used in developing tools to efficiently evaluate sex-specific responses in early life stage fish exposed to EDCs. These tools will help us better understand the mechanisms regulating sex differentiation in fathead minnows and how EDCs may alter these processes.

**MP055 Comparison of genotoxic potential and related mechanisms of various bisphenols using DNA-repair-deficient chicken DT40 cell lines** S. Lee, Seoul National University / School of Public Health, Seoul

National University; K. Choi, Seoul National University / School of Public Health. Bisphenol A is a common ingredient of polycarbonate and epoxy resins which have been widely used in plastic food containers, medical devices and toys. Over 2.2 million tons of BPA was produced worldwide as of 2009. BPA has been detected in various food items and human serum up to few hundreds ng/L, and suspected for adverse health effects such as reproduction damages, developmental changes, genotoxicity and carcinogenesis. Because of such concerns, more alternative bisphenols are expected to be used. In the present study, we investigated the genotoxicity potential and mechanisms of BPA and alternative bisphenols such as Bisphenol AF, bisphenol AP, bisphenol B, bisphenol F, bisphenol M, bisphenol P, bisphenol S and bisphenol Z using genetically modified chicken DT40 cell. Responses of a panel of isogenic DT40 mutants, each defective in one of the major DNA damage repair mechanisms, which include base excision repair (*Pol $\beta$ <sup>-/-</sup>*), nucleotide excision repair (*XPA<sup>-/-</sup>*), homologous recombination (*Rad54<sup>-/-</sup>*), non-homologous end-joining (*Ku70<sup>-/-</sup>*), and translesion DNA synthesis (*REV3<sup>-/-</sup>*) were evaluated after exposure to each of bisphenols. The cell viabilities of each mutants and the wild-type were measured and only the noncytotoxic doses that resulted in < 80% decrease of cell viability in wild-type cells were evaluated for genotoxicity. BPP showed greatest genotoxic potential than the other bisphenols. The *Rad54<sup>-/-</sup>* mutant cell showed hypersensitivity to all the test bisphenols, suggesting that the genotoxicity of bisphenols involves DNA damages which can be repaired by homologous recombination. Bisphenols may cause double strand breaks that can be repaired by homologous recombination. The results of cell viability were compared with those of chromosomal aberration assay in the same mutant cell.

**MP056 Microarray analysis in the ovary of Queen conch (*Strombus gigas*) reveals reproduction and stress are impacted in environments with high tributyltin** R. Kan, C. Titley-O'Neal, University of New Brunswick; D. Spade, Y. Zhang, University of Florida; C.J. Martyniuk, University of New Brunswick / Dept. of Biology/Canadian Rivers Institute, University of New Brunswick / Biology; N. Denslow, University of Florida; B. MacDonald, University of New Brunswick. The Queen conch (*Strombus gigas*) is a threatened species due to overharvest throughout much of its range prior to the 1980s. Conch populations are declining or stagnant despite harvest protections due to anthropogenic impacts that include pollution from boating activities. In the British Virgin Islands (BVI), individuals from local populations have exhibited imposex, a condition in which there is a superimposition of male organs (penis and or vas deferens) in gonochoristic female gastropods. Studies in the BVI suggest that tributyltin (TBT), a compound used as an antifouling paint, is correlated with an increased incidence of imposex. However, the direct mechanisms leading to imposex are not well understood. The present study utilized an 8 x 15K Queen conch microarray to measure the response of the ovarian transcriptome in conch inhabiting polluted environments with high TBT in the BVI. The polluted sites, Road Harbour (RH) and Trellis Bay (TB), are harbours with high boating activity while the reference sites, Guana Island (GI) and Anegada (AN), are areas with low boating activity. At RH and TB, there were 754 and 898 probes differentially expressed in the ovary from the control (GI) site ( $P$ -value 0.05,  $E$ -value <  $10^{-4}$ ). There were few transcripts (< 10%) that were affected at both sites, suggesting that there are additional environmental factors influencing gene expression patterns. When considering both RH and TB together in comparison to GI, functional enrichment showed that the biological processes and molecular functions of calcium ion binding, immune response, and negative regulation of cell proliferation were over represented in the polluted sites. Sub-network enrichment analysis revealed that transcripts involved in the biological processes of general metabolism, immune, lipid metabolism, and stress were affected in polluted environments. The disruption of lipid metabolism may provide some insight into the induction of imposex due to the activation of the retinoic X receptor by TBT. Furthermore, stress appeared to be prevalent in Queen conch collected from RH rather than those collected from TB. This may correspond to the higher TBT load at RH. Our study shows that multiple Queen conch pathways are affected by an environment that experiences heavy boating activity in the BVI. However, a limitation is to directly link changes at the transcriptomics level to high TBT levels in the harbour.

**MP057 Analysis of oil and dispersants adverse effects on the sheepshead minnows** N. Vinas, Mississippi State University; K.J. Kroll, University of Florida / Physiological Sciences; L. Escalon, US Army Engineer Research

and Development; T. Habib, BTS; N.D. Denslow, University of Florida / Department of Physiological Sciences and Center for Environmental and Human Toxicology, University of Florida / Dept. of Physiological Sciences &. The Deep Water Horizon oil spill resulted in the release of over 200 million gallons of crude oil into the waters of the Gulf of Mexico. More than 2 million gallons of the dispersants Corexit EC9500A and Corexit EC9527A were applied either to surface waters or to deep water in order to disperse the oil in an attempt to accelerate oil degradation. Crude oil hydrocarbons and surfactants have been shown to cause structural damage in the gill, the major route of pollutant uptake in aquatic organisms affecting gas exchange processes and limiting oxygen transfer, facts that could lead the organisms to experience hypoxic conditions. Using an ecologically relevant species, the sheepshead minnow (*Cyprinodon variegatus*, SHM), we have shown that exposures to mixtures were more toxic than the dispersant and oil individually. In this experiment, male SHM were exposed for four days to water-accommodated fractions (WAF) of oil (0.5 ppm), Corexit EC9500A (0.05 ppm), Corexit EC9527A (0.05 ppm), and mixtures of oil and each one of the dispersants (0.5ppm oil/0.05 ppm dispersant) in our laboratory. Mixtures of dispersants and oil were designed to mimic proportions used in the Gulf during the spill. Exposure to just 3 ppm of Corexit EC9527A resulted in 100% mortality. A mixture of 0.5 ppm Corexit 9527/ 5 ppm oil also resulted in 100% mortality. We analyzed gene expression changes in the liver and testis of exposed SHM using a commercially available 15,000-gene microarray (EcoArray, Alachua, FL). In the liver, Corexit EC9500A and oil were the least toxic compounds, while both mixtures and Corexit EC9527A were similar in toxicity and had the most effects. Interestingly, although Corexit EC9500A is considered to be much less toxic than Corexit EC9527A, our results show that when combined with the WAF their toxicity is similar. Furthermore, 76% of the differentially expressed genes were common between the two mixtures, indicating the high similarity in toxicity of both Corexits when mixed with the oil. This common response was related to pathways such as oxidative stress, hypoxia, cardiac hypertrophy, liver proliferation, and dopamine receptor signaling. Many of these effects are in accordance with previously described effects such as hypoxia, cardiovascular abnormalities, cardiac dysfunctions or neurotoxicity. These observations suggest that dispersants and oil could pose a greater threat to the marine ecosystem than previously suggested.

**MP058 Chemical flame-retardant toxicity and accumulation in *Daphnia magna*** L.D. Scanlan, U.C. Berkeley / Molecular Toxicology, U.C. Berkeley / graduate student; K. Dailey, U.C. Berkeley; K. Douglas, H. Stapleton, Duke University; C. Vulpe, University of California, Berkeley / Nutritional Sciences and Toxicology. Chemical flame-retardants (FR) are commonly used in furniture foam and fabric, building insulation and electronics casings. These chemicals are not covalently bound to the items in which they are used, so they leach into the environment where there is potential for toxicity. Firemaster550® (FM550) is a chemical formulation used as a replacement for penta-brominated diphenyl ether (penta-BDE), which was banned from use because of toxicity, persistence and accumulation. FM550 components have been detected in many different kinds of animals including sea mammals and in indoor house dust. In this work, we first determined the acute 48-hour LC<sub>50</sub> of FM550, its components, and penta- and octa-BDE on *Daphnia magna*, an eco-indicator used in water quality assessment. We then performed gene expression microarray assays to determine differential expression in daphnids exposed to each FR. We also analyzed *Daphnia magna* exposed to two concentrations of FM550 to determine if the chemicals accumulated in their tissues. We found that each FR caused a unique expression pattern in the daphnids. Penta-BDE was the most toxic FR, while octa-BDE was the least. Furthermore, daphnids accumulated FM550 after a 48-hour aqueous exposure to 48.6 µg/L. These results may indicate that *Daphnia* has the potential to bioaccumulate FM550 in aquatic ecosystems. Results also indicate that hydrophobic compounds effect *Daphnia* in unique ways, as is evident from the chemicals' different gene expression profiles. Further work includes chronic toxicity assays, an analysis of the toxicity of Bis(2-ethylhexyl) phthalate, a plasticizer with similar structure to one of FM550's brominated components, and effects on the lipidome of exposed *Daphnia*.

**MP059 Evaluation of the Micronucleus Formation After Exposure to Environmentally Relevant Concentrations of Atrazine Herbicide** R.G. Borelho, University of Sao Paulo / Ecotoxicology; S.H. Monteiro, L.A. Maranhão, P. Alves, G. Moura Andrade, V. Tornisielo, Center for Nuclear

Energy in Agriculture/University of Sao Paulo. Atrazine is one of the most used herbicide in the cultivation of sugar cane in the region of Piracicaba River (São Paulo, Brazil) which probably can be contaminated by this molecule. The goal of this work was to monitor this herbicide in the surface water in six points of this water course during one year through Liquid Chromatography Tandem Triple Quadrupole Mass Spectrometry (LS-MS/MS) and to exposure the concentrations found to the fish *Danio rerio* to verify the micronucleus formation. First of all a rapid method was developed and validated for determination of atrazine in surface waters. The method consists in filtering the water in 0.45 µm filter followed by injection into Liquid Chromatography Tandem Triple Quadrupole Mass Spectrometer (LC-MS/MS). The transitions monitored were: 216.0/103.9 and 216.0/173.9. The limits of detection and quantification (LOD and LOQ) were 0.07 µg·L<sup>-1</sup> and 0.10 µg·L<sup>-1</sup> respectively. During the sampling period were found concentrations of atrazine between 0.11 to 1.92 µg·L<sup>-1</sup> that is below of the Brazilian environmental legislation that is 2 µg·L<sup>-1</sup>. Thus, *D. rerio* were exposed to 1.0; 1.5 and 2.0 µg·L<sup>-1</sup> of this product plus control group (maintenance water) for 4 days in beakers (2L) with aeration system. Each treatment consisted of 2 replicates with 7 organisms in each. During the test no feed was provided for the fish and the test solutions were not renewed. After the exposition period six animals (n = 6) of each treatment were anesthetized and had its tail sectioned to obtain blood. Blood was dripped on microscopic slides and fixed in methanol for 10 minutes and then stained with Giemsa 5%. For each animal was counted 1000 cells that were observed in microscope and photographed in case of appearance of micronucleus and erythrocytic nuclear abnormalities. Micronuclei formation and erythrocytic nuclear abnormalities were observed in all concentrations (p < 0.05) and in the lower concentrations respectively. We concluded that Environmentally relevant concentrations of atrazine showed be mutagenic and genotoxic to *D. rerio* and probably lower concentrations can have the same effect since they are present in the water. Thus, new test should be done to confirm this information.

**MP060 Gene expression profile, lipid peroxidation and oxidative DNA damage in carp fish following exposure to hypoxic and subsequent recovery conditions** S.A. Mustafa, University of Plymouth / Biomedical and Biological Sciences. S A Mustafa, S J Davies, A N Jha School of Biological Sciences, University of Plymouth, UK Email: sanaa.mustafa@plymouth.ac.uk In fish a complex set of physiological and biochemical strategies are employed to cope with environmental stress including hypoxic stress, underlying mechanism of which could be expression of certain key genes. In order to probe the hypotheses that hypoxia induces oxidative stress and this stress could be manifested in varieties of way, *Cyprinus carpio*, a model fish species, were chronically exposed to hypoxic condition (1.8±0.6 mg l<sup>-1</sup>) for 21 days and subsequently allowed to recover under normoxic condition for 7 days. At the end of these exposure periods maximising the use of biological samples and adopting an integrated approach several endpoints were evaluated at different level of biological organisation. These included determination of (a) expression of hypoxia inducible factor 1 (HIF-1α) gene, DNA repair genes (xrcc1 and ogg1) using RT-PCR in liver samples (b) oxidative DNA damage in erythrocytes (using modified Comet assay employing bacterial enzymes: Fpg and Endo-III), (c) lipid peroxidation in liver samples by measuring the MDA production using the 2-thiobarbituric acid (TBARS test) (d) histopathological changes in gills. The results suggested that the expression levels of HIF-1α in response to hypoxia were significantly higher compared to concurrent normoxic controls, which reverted to control values within 7days exposure to normoxic condition (P < 0.05). The expression of xrcc1 and ogg1 were significantly increased under both hypoxic and recovery conditions. Interestingly, the highest rate of oxidative DNA damage occurred when the fish were kept under hypoxic conditions followed by a rapid increase of the oxygen concentration (recovery period for 7 days) compared to fish maintained under normoxic condition as concurrent controls. Hypoxic groups showed significantly increased values for TBARS levels (by ~ 2 fold) compared to both normoxic and recovery groups. Histopathological changes revealed damage in gill tissue under both hypoxia and recovery stages. Taken together, the results suggest that exposure to hypoxia resulted in the induction of biological damage at different levels of biological organisation.

**MP061 Differential gene expression in rainbow trout (*Oncorhynchus mykiss*) exposed to Randle Reef sediment** J.P. Sherry, T. Neheli, Environment Canada / Water Science & Technology Directorate; D.E. Crump, Environment Canada / National Wildlife Research Centre, Environment



Canada / Canadian Wildlife Service; S.W. Kennedy, Environment Canada, University of Ottawa / Department of Biology, University of Ottawa / Department of Biology, Environment Canada / National Wildlife Research Centre, Environment Canada. Hamilton Harbour was designated an Area of Concern (AOC) by the International Joint Commission in 1985. For that reason, there is an ongoing Remedial Action Plan (RAP) to restore the harbour's beneficial uses. Sediment in the Randle Reef (RR) area of Hamilton Harbour is highly contaminated with polycyclic aromatic hydrocarbons (PAHs) and heavy metals. We report the use of Fluorescent RNA Arbitrarily Primed Polymerase Chain Reaction (FRAP-PCR) to develop a molecular fingerprint in juvenile rainbow trout that were exposed to RR sediment. FRAP-PCR enabled us to identify 42 unique genes that were differentially expressed in livers of fish exposed in the laboratory to RR sediment compared to fish exposed to Lake Ontario water. A 20-gene subset, all of which were confirmed by qPCR, was selected as a fingerprint for use in the diagnosis of exposure to RR contaminants. CYP1A and Vtg both of which are known to change expression upon exposure to PAHs were up-regulated by exposure to RR sediment. Pathways analysis showed that exposure to RR sediment altered the expression of genes within multiple categories including cell adhesion, cell morphogenesis, DNA synthesis, and immune response. Pathways analysis also enabled us to explore links between the modulated transcripts and potential health effects. In the next phase we propose to use the diagnostic fingerprint to track the efficacy and progress of the proposed remediation of RR from the perspective of fish in the adjacent waters.

**MP062 Toxicogenomic approach to determine contaminant exposure using Hornyhead Turbot (*Pleuronichthys verticalis*)** D.E. Vidal-Dorsch, S.M. Bay, Southern California Coastal Water Research Project; M.C. Brown-Augustine, University of California- Berkeley / Department of Nutritional Science and Toxicology; C. Vulpe, University of California, Berkeley / Nutritional Sciences and Toxicology. Previous work in our laboratories has demonstrated that gene microarrays from model and non-model aquatic organisms (fathead minnows, *Pimephales promelas*, the amphipod, *Eohaustorius estuaris*, and the water crustacean, *Daphnia magna*) produce gene-exposure profiles related to specific chemical classes or environmental chemical mixtures. New monitoring tools that perform rapid screening of contaminant effects in aquatic ecosystems need to be developed and validated before they can be incorporated into regional assessments of environmental quality. We are currently developing additional tools using the marine flatfish hornyhead turbot (*Pleuronichthys verticalis*), an indigenous benthic species from southern California. To investigate exposure effects, we are producing a high-density microarray with sequences obtained using Next-Generation techniques. This array will be used to generate gene expression profiles after laboratory exposures to estradiol, ammonia, cadmium, and effluents (secondary treated and primary treated). In addition, we will obtain gene profiles from fish collected in areas near municipal wastewater discharges and reference sites. Our data will assess the usefulness of a biomonitoring indicator using hornyhead turbot (*Pleuronichthys verticalis*) transcriptomic data from a microarray.

**MP063 Modulatory effects of marine pollutants on whole cytochrome P450 (CYP) genes in the marine medaka, *Oryzias melastigma*** J. Lee, Hanyang University / Dept of Chemistry, Hanyang University Graduate School / Dept of Chemistry; B. Kim, J. Rhee, Hanyang University. The cytochrome P450 superfamily is a large and diverse group of enzymes. P450 gene expression, protein levels and P450 mediated metabolism of xenobiotics are induced by PAHs in diverse aquatic species. Thus, regulation of P450 enzyme activity may play a central role in the adaptation of aquatic animals to environmental pollutants. To understand the effects and fates of marine pollutants, we identified the cytochrome P450 (CYP) gene superfamily in the marine medaka, *Oryzias melastigma*. We searched for CYP genes in local genome databases for the marine medaka which were constructed and sequenced by Next Generation Sequencing (NGS) technologies. Finally, we investigated functional 70 CYP genes in *Oryzias melastigma*. To determine the placements of CYP genes, we performed the phylogenetic analysis with those of diverse organisms using maximum likelihood and Bayesian method. The same clade of CYP genes were separated and clustered into the well-known vertebrate CYP clans, CYP 2, 3, 4, and mitochondrial clans, respectively. We analyzed the specific expression patterns of CYP transcripts in developmental and different sexual stages. Expression patterns of CYP transcripts were also analyzed by real-time RT-PCR after exposure to marine pollutants (e.g. benzo[a]pyrene and water-accommodated fraction (WAF) of

crude oil). Differentially modulated expression patterns of CYP transcripts suggest that these CYPs would be involved in defenses and detoxification mechanisms against marine pollutants. Overall, this is the first report to characterize the whole CYP gene superfamily expressed in the marine medaka, *Oryzias melastigma*. This study would be helpful to expand the knowledge on their innate roles in xenobiotic metabolism and physiology of aquatic species.

**MP064 Mitochondrial DNA as a target of environmental toxicants** J.N Meyer, Duke University / Nicholas School of the Environment; M.C. Leung, Duke University / Nicholas School of the Environment, Duke University / graduate student; J. Rooney, A. Ji, I. Ryde, I. Ryde, Duke University; A.S. Bess, Duke University / Nicholas School of the Environment. Mitochondrial DNA (mtDNA) has received less attention than nuclear DNA (nDNA) as a target for genotoxins, but a growing body of evidence suggests that mtDNA damage may be important both for health and as a biomarker for exposure. I will briefly review mtDNA biology and what is known about mtDNA environmental genotoxicity, and then present recent research on the fate and effects of persistent mtDNA damage. mtDNA is more sensitive than nuclear DNA to many common genotoxins, and lacks some repair pathways that are present in the nucleus. In particular, helix-distorting adducts formed after exposure to environmentally important genotoxins such as ultraviolet C (UVC) radiation and some polycyclic aromatic hydrocarbons and mycotoxins are repaired in the nuclear genome by nucleotide excision repair. However, nucleotide excision repair does not occur in the mitochondria, so that the fate of such DNA damage in the mitochondrial genome is unknown. The effects of such persistent damage are also unknown; we hypothesized that the effects of such damage would be particularly important after early life stage exposure since mtDNA copy number is lowest at that time. Using the nematode model *Caenorhabditis elegans*, we found that UVC-induced photodimers result in lower levels of mtDNA-encoded mRNAs, decreased ATP levels, decreased oxygen consumption, larval developmental arrest, and neurodegeneration. Furthermore, UVC-induced mtDNA damage is slowly removed in a process dependent at least in part on mitochondrial fusion, fission and autophagy. Mutations in mitochondrial fusion and autophagy genes exacerbate the larval arrest, suggesting a potent gene-environment interaction in which the effects of mtDNA damage caused by environmental agents are exacerbated by decreased mitochondrial fusion and autophagy.

**MP065 Effect of Anthropogenic Contamination on Genetic Diversity in Mediterranean Mussels from the Strait of Istanbul** C. Theodorakis, Southern Illinois University Edwardsville / Department of Biological Sciences and Environmental Sciences Program, Southern Illinois University Edwardsville / Environmental Sciences, Southern Illinois University / Environmental Sciences; K. Schramm, GSF Helmholtz Zentrum / Institute of Ecological Chemistry; O. Okay, Istanbul Technical University; M. Meyer, Southern Illinois University Edwardsville / Biological Sciences. The objective of this study was to determine the effects of urban contamination on genetic diversity of Mediterranean mussels (*Mytilus galloprovincialis*) from the Strait of Istanbul, Turkey. Mussels were collected from two reference sites and five other sites with varying degrees of contamination within a 50 km stretch of shoreline in or near Istanbul. Sections of the cyclooxygenase subunit III (COX III) gene and the D-loop were PCR-amplified from mitochondrial DNA and sequenced. Sequences were analyzed by examining genetic diversity within and among sites (nucleotide and haplotype diversity indices, Nei's genetic distance), phylogeographic patterns (minimum spanning trees, Mantel tests), partitioning of genetic diversity (AMOVA), asymmetric migration patterns, and spatial structuring (Bayesian analysis). Genetic diversity of the D-loop was represented by both nucleotide substitutions and complex insertion/deletion patterns. Both loci revealed two major clades, one of which was significantly distinct from any other published sequences from individuals of this species collected in the Mediterranean. Both loci also revealed fine-grained genetic structuring and genetic differentiation among sites. Finally, both loci revealed that the genetic diversity of the contaminated sites was greater than that of the reference sites, which may have been due to mutations or alterations in gene flow. These findings are significant because it 1) provides further evidence that anthropogenic disturbance can increase genetic diversity of impacted populations, 2) suggest a unique evolutionary history for one of the major clades, 3) demonstrates that fine-grained genetic structuring may occur over short distances in marine species with pelagic larvae, and 4) it is one of the first studies that demonstrates

possible anthropogenic impacts on genetic diversity in a marine species with a pelagic dispersal stage.

**MP066 Acute toxicity of Iranian Heavy crude oil to the Rockfish (*Sebastes schlegeli*); feeding disrupts the hepatodetoxification and immune systems** H. Kim, Oil and POPs Research Group, Korea Ocean Research and Development Institute, Korea; Y. Che, R. Addison, W. Shim, J. Jung, Oil and POPs Research Group, Korea Ocean Research and Development Institute. Oil spilled into marine environments induces the hepatodetoxification system and disrupts immune function in fish (Reynaud and Deschaux, 2006). However, the mechanism of toxicity to the immune system has not been fully investigated. Changes in the immune system may affect disease susceptibility in fish (Zelikoff, 1998). In this study, we describe the effects of spilled crude oil from the “*Hebei spirit*” (Iranian Heavy Crude Oil in gelatine capsules) on juvenile rockfish. Effects on multiple hepatodetoxifying enzymes (Cytochrome P4501A and GST) and on the immune responses including interleukin-1 $\beta$  (IL-1 $\beta$ ), granulocyte colony-stimulating factor (G-CSF) and Cathepsin L (Cat. L) were measured in liver, spleen and kidney. From 12 h after treatment, the oil-fed groups had slightly higher concentrations of bile fluorescent metabolites and CYP1A expression than the control group in liver and spleen. Cat. L and G-CSF mRNA also increased significantly in the early stages of exposure (from 12h to 48h after exposure) but then decreased to the levels in the control group, or lower. Therefore, it is suggested that innate immune response of oil-fed fish induced sensitively at initial stage due to the IHCO exposure but several immune-related markers may decrease to less than that of control group after initial stage of response.

**MP068 Development of two small fish species, *Pimephales promelas* and *Cyprinodon variegatus*, as model organisms for immunotoxicity** M.K. Sellin Jeffries, Miami University / Department of Zoology, Miami University; B.A. Arivett, S.E. Fiester, Miami University / Department of Microbiology; D.D. Coffey, L.M. Thornton, Miami University / Department of Zoology; A.W. Smith, Miami University; L.A. Actis, Miami University / Department of Microbiology; J.T. Oris, Miami University / Department of Zoology. To adequately assess the environmental risks associated with exposures to aquatic pollutants, the effects of contaminant exposures on immune function should be considered. To date, only a handful of fish species (e.g., *Oryzias latipes*, *Onchorynchus mykiss*) have been utilized in studies investigating the impacts of toxicant exposures on immune function. The goal of the current study was to develop the fathead minnow (FHM, *Pimephales promelas*) and the sheepshead minnow (SHM, *Cyprinodon variegatus*) as model organisms for assessing immunotoxicity in freshwater and estuarine fish, respectively. There is a paucity of information regarding basic immune function in FHMs and SHMs; therefore, the first objective of this study was to characterize basic aspects of immune function in each species. This was accomplished by measuring 1) leukocyte numbers, 2) differential mRNA expression of immune-related genes in blood, spleen, liver and kidney tissue, 3) serum IgM levels, 4) plasma lysozyme activity and 5) pathogen resistance in adult FHMs and SHMs with no known history of pathogen or contaminant exposure. The second objective of this study was to evaluate the effects of a model immunotoxicant, benzo[a]pyrene (BaP), on immune function in FHMs and SHMs. Fish exposed to BaP were intraperitoneally-injected with 200  $\mu$ g/g body weight BaP, while the vehicle control fish were injected with corn oil. Forty-eight hours after the injections, a subset of fish from each group was sacrificed and tissues were collected for determination of leukocyte numbers, mRNA expression, IgM levels and lysozyme activity. The remaining fish were subjected to a pathogen resistance trial in which the mortality of minnows intraperitoneally-injected with an LD<sub>30-50</sub> dose of bacteria (i.e., *Yersinia ruckeri* for FHMs and *Vibrio anguillarum* for SHMs) was monitored for 10 days following the pathogen exposure. These results of this study demonstrate that both the FHM and SHM are suitable model organisms for use in immunotoxicity studies.

**MP071 PCB-126 exposure lowered antimicrobial peptide secretion in juvenile northern leopard frogs [*Lithobates (Rana) pipiens*]** T.L. Cary, University of WI – Madison / Forest and Wildlife Ecology, University of WI-Madison / Zoology, University of WI-Madison / Forest and Wildlife Ecology; J.D. Pask, Vanderbilt University Medical Center / Pathology, Microbiology and Immunology; L.A. Rollins-Smith, Vanderbilt University Medical Center / Pathology, Microbiology and Immunology, and of Biological Sciences; W.H. Karasov, University of Wisconsin / Zoology, and

of Forest and Wildlife Ecology. Chytridiomycosis, an emerging infectious disease of amphibians caused by the fungus *Batrachochytrium dendrobatidis* (*Bd*), has been implicated in declines of amphibian populations world-wide. In addition to disease, another factor threatening global amphibian populations is environmental contamination. Measures of immune function provide a way to analyze how contaminant exposure may be linked to disease susceptibility. In the case of *Bd*, antimicrobial peptides (AMPs) secreted onto the skin of amphibians are thought to defend against the fungus, thus acting as a first-line innate immune strategy. We hypothesized that larval exposure to PCB-126, an environmentally persistent organic contaminant, would decrease AMPs secreted by *Rana pipiens*. In order to obtain newly metamorphosed froglets carrying a body-burden of PCB-126, tadpoles were fed a diet containing 0.37, 1.2, or 5.0 ng PCB-126/g. A control group was fed the same diet, but without PCB-126 (0.0 ng/g). Post-metamorphic (10.3  $\pm$  1 [S.E.M.] weeks) frogs were injected with amphibian phosphate buffered saline (APBS) or norepinephrine-HCl dissolved in APBS and placed in buffer to collect secreted skin peptides. The buffer was acidified, and the peptides were enriched by passage over C18-Sep-Paks to obtain hydrophobic peptides which include the AMPs. AMPs were quantified using a bradykinin based peptide assay. Additionally, skin peptides were analyzed using matrix-assisted laser desorption time-of-flight mass spectrometry to determine any differences in type of AMPs secreted between control and PCB-treated frogs. Control frogs secreted 593  $\pm$  101  $\mu$ g/g body weight (BW) ( $n$  = 10) skin peptides, while frogs exposed to 0.37, 1.2, or 5.0 ng PCB-126/g had 336  $\pm$  43 ( $n$  = 11), 378  $\pm$  62 ( $n$  = 10), and 365  $\pm$  56 ( $n$  = 10)  $\mu$ g/g BW skin peptides, respectively. However, this decrease was not statistically significant (1-way ANOVA,  $F_{3,37}$  = 2.47,  $p$  = 0.0769). Additionally, the suite of AMPs secreted by frogs in all treatment groups was similar, indicating that the type of AMP secreted was not affected by PCB exposure. If there is a minimum threshold of AMPs necessary to be protective of *Bd* infection in juvenile *R. pipiens*, lowered AMP levels due to contaminant exposure may make froglets more susceptible to *Bd*. Supported by UW Sea Grant Institute (grant number NA16RG2257, project number R/EH-2).

**MP072 The immunologic effects of waterborne oil sands-derived naphthenic acids on rainbow trout** L. Leclair, / Environmental Sciences; N.S. Hogan, University of Saskatchewan / Toxicology Centre; B. Koellner, Friedrich Loeffler Institute; G.Z. MacDonald, Canadian Rivers Institute, University of Prince Edward Island; M.R. Van den Heuvel, Canadian Rivers Institute, University of Prince Edward Island. Naphthenic acids are a major organic constituent in waters influenced by oil sands contamination. With increased diseases in fishes being associated with oil-sands influenced waters, there is interest surrounding the potential of naphthenic acids as an immunotoxicant. The lack of availability of naphthenic acid standards representative of that found oil sands required the extraction of naphthenic acids from 17-year-old tailings waters. High resolution mass spectrometry indicated that the extracted naphthenic acids were at least 91% representative of classical naphthenic acid structures. The median lethal threshold of the extracted naphthenic acids was 15 mg/L. For immune experiments, rainbow trout were exposed to 0, 1, and 8 mg/L naphthenic acids in a flow through system in two separate trials. After 7 d of exposure, a portion of the rainbow trout were sampled for immune parameters. To assess the immunologic response blood, spleen, gill and head kidney samples were taken at the end of the exposure and the constituent leukocytes, T-lymphocytes, B-lymphocytes, thrombocytes and myeloid cells were counted and typed via flow cytometry analysis using fluorescent antibodies specific for those cell types. Total cell counts were done on each tissue using flow cytometry in conjunction with a fluorescent dye, DiOC6. The remainder of the trout in each experiment were injected with inactivated *Aeromonas salmonicida* and held in clean water for 21 d and subject to similar lymphatic cell evaluation in addition to evaluation of antibody production by ELISA. Naphthenic acid responses were compared to a positive control, benzo[a]pyrene, a known immune suppressor. Naphthenic acids had no significant impacts on total leukocytes, any of the four leukocyte types, or on erythrocytes in blood. There were significant decreases in total leukocytes, thrombocytes and B-cells in spleen, but only as compared to the low dose, which was elevated over the control. Unlike the PAH benzo[a]pyrene, that profoundly depletes splenic and blood B-leukocytes, naphthenic acids appear to show a more complex response of stimulation and depression, and thus do not likely act by the same mechanism.



**MP073 Timing of Thymocyte Cell Death Induction and Receptor Usage by DES and Methoxychlor Metabolite, HPTE, during embryonic exposure** C. Broussard, The University of La Verne, University of La Verne / Biology, University of La Verne / Professor of Biology; A. Lim, University of La Verne / Biology; Z. Muscato, C. Zambrano, S. Moeller, University of La Verne; L. Leung Liu, The University of La Verne, University of La Verne. A number of chemicals prevalent in the environment have been identified as estrogenic endocrine disrupting chemicals (estrogenic EDCs). Studies have shown that estrogenic EDCs not only alter the reproductive system, the brain, and behavior, but may also alter immune responsiveness. Previously, we investigated the effects of two estrogenic EDCs, diethylstilbestrol (DES) and hydroxyphenyl-trichloroethane (HPTE), on embryonic thymocyte development and found that both appear to induce cell death and alteration of differentiation. The assumption has been that estrogenic EDCs, eg. BPA, DES, nonylphenol, and pesticides like methoxychlor and its metabolite HPTE, utilize estrogen receptors to mediate their effects. In order to determine whether and which estrogen receptors DES and HPTE may use, we conducted several experiments. First, we did a time course of treatment to determine how quickly DES and HPTE induced death in developing embryonic thymocytes. Rapid induction of apoptosis would suggest a receptor-initiated signaling cascade, whereas slow induction would imply a transcriptionally mediated pathway. Second, we used an inhibitor of the classical estrogen receptor alpha and, in separate experiments, an inhibitor of GPR30, a nonclassical estrogen receptor, to block any interactions DES or HPTE might have with ER alpha or GPR30, respectively. The rescue of apoptosis and alteration of embryonic thymocyte differentiation by one or both of the inhibitors would indicate which receptor was primarily responsible for the estrogenic EDCs adverse effects. Third we stimulated embryonic thymocytes after exposure to estrogenic EDCs in FTTC, to determine if thymocytes matured in this environment were functional. We present the findings of these experiments and propose a mechanism for estrogenic EDC action in differentiating embryonic thymocytes. This material is based in part upon work supported by the National Institute of Environmental Health and Safety (NIEHS) under Grant No. 1R15ES017345-01. Any opinions, findings and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the NIEHS.

**MP074 Evaluation of the Ecotoxicological Effects of Dexamethasone** J.L. Ribas, I.C. Guiloski, Federal University of Paraná / Pharmacology; G.L. Galvan, Federal University of Paraná / Genetic; E.M. Reynaldo, Hospital da Cruz Vermelha Brasileira filial do Paraná / Research Center; M.M. Cestari, Federal University of Paraná / Genetic; A. Zampronio, H.C. Silva de Assis, Federal University of Paraná / Pharmacology. Steroid hormones have been found in water bodies. In this study, toxic effects of an environmental water contaminant dexamethasone (DEX) were evaluated. Male Swiss mice received water containing DEX (0.03-3.0µg/L) for 14 days as the only source of drinking water. Control group received only water. Mice were then killed and biochemical (aspartate aminotransferase [AST], alanine aminotransferase [ALT], total protein [PT], albumin [ALB], globulin [GLOB], creatinine [CREAT], sodium [Na], potassium [K], calcium [Ca] and creatine phosphokinase [CPK]), hematological (Erythrocyte, hemoglobin, hematocrit, leukocyte and platelet) and immunological biomarkers (cell migration induced by carrageenin [Cg] and NO production induced by lipopolysaccharide [LPS] at 1 to 10000ng/mL in macrophages) were carried out. Additionally, total weight, abdominal fat and adrenal weight were determined. One Way ANOVA followed by Bonferroni's post hoc test was applied. The 3.0µg/L DEX-treated group showed an increase in abdominal fat compared to the control group. The adrenal weight had a significant increase in all the treated groups. Both AST and ALT increased significantly at 3.0µg/L DEX-treated group, as well as GLOB and K. CPK increased in all concentrations of DEX tested. Other biochemical parameters did not show statistically significant differences among the groups. In relation to hematological biomarkers, DEX, at lower concentrations (0.03-0.3µg/L), reduced the number of leukocytes while at a higher concentration (3µg/L) this number was increased. The number of resident peritoneal cells did not differ between the test and control groups, but there was a significant reduction in the cell migration elicited by Cg in the all DEX-treated groups due to a significant decrease in the migration of polymorphonuclear cells. Basal NO synthesis by macrophages from DEX-treated animals was significantly lower when compared to the control group. LPS-stimulated NO production was reduced in macrophages from 3µg/L DEX-treated mice compared

to control macrophages, independently of the concentration of LPS used to stimulate the cells. The results suggest that a constant exposure of organisms to dexamethasone present in water can lead to potential toxic effects, including biochemical, hematological and immunological changes, showing that more attention must be given to the proper disposal of pharmaceutical products in water.

**MP075 The Effects of Diethylstilbestrol on Maturation and Differentiation of Sex Specific Embryonic C57BL/6 Thymocytes in Organ Culture** C. Zambrano, University of La Verne / Department of Biology; C. Broussard, The University of La Verne, University of La Verne / Biology, University of La Verne / Professor of Biology. The endocrine system consists of a network of hormone producing glands. Hormones are released in low doses and serve as chemical messengers that regulate many of the body's functions, including the immune system. Endocrine disrupting chemicals (EDCs) are substances, which interfere with the natural occurring endocrine system of the body. These chemicals display hormone like properties or interfere with hormone activity, which may disrupt the development of the immune system. It has been demonstrated in studies of neonatal and adult systems that sex is also a variable in the effects of EDCs. One such EDC is diethylstilbestrol (DES), a synthetic estrogen that serves as a model for other EDCs. DES was once prescribed during pregnancy to prevent complications and was later discovered to cause endocrine disruption. It has been reported that females exposed to DES *in utero* may have an increased risk of developing a rare form of vaginal cancer at a young age. Reproductive tract abnormalities have been reported in both females and males of second and third generation offspring of DES mothers. Along with reproductive effects, there is evidence that developmental exposure to DES can alter the functioning of the immune system. Previous studies shown that DES impacts developing thymocytes, yet no studies have examined how DES effects on thymocyte development differ in male and female embryos. The aim of this study was to determine whether DES effects on the different subpopulations of developing embryonic T cells differ based on sex of the embryo. PCR-amplification of a Y chromosome gene was used in sex identification. An *ex vivo* assay was used to examine the initial development of T cells and an *in vitro* assay with phytohemagglutinin stimulation examined mature T cells. Thymii were extracted from C57BL/6 mice embryos at 16 to 18 days of gestation. Our results indicate a decrease in T cell viability of both sexes in a dose dependent manner, and effects on the different subpopulations were also observed. These findings suggest that DES affects normal development and survival rate of both male and female embryonic T cells. This material is based upon work supported by the National Institute of Environmental Health and Safety (NIEHS) under Grant No. 1R15ES017345-01. Any opinions, findings and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the NIEHS.

**MP076 BIMS: A database of baseline physical, chemical, and ecological conditions in the Gulf of Mexico prior to the Deepwater Horizon accident** A. Fogg, M. Henning, M. Meaders, J. Nicolette, ENVIRON International Corporation. In Natural Resource Damage Assessment, accurate characterization of an ecosystem's baseline conditions is critical to the accurate quantification of injury. Baseline is the condition of the natural resources and services that would have existed had an incident not occurred. It is not possible to accurately quantify natural resource injuries from the Deepwater Horizon release in the Gulf of Mexico without a thorough understanding of baseline conditions. It is also important to capture long-term trends associated with physical, chemical, and biological stressors affecting baseline conditions. Adequately characterizing baseline conditions of the Gulf of Mexico is uniquely challenging, given the large geographic scale, diversity of natural resources and the services they provide, and immense volume and diversity of data available. Further complicating matters is the fact that baseline conditions are not static; conditions may cycle seasonally or may decline or improve over time for reasons completely unrelated to the release. We are developing a Baseline Information Management System (BIMS) that is robust and flexible enough to meet these challenges. We describe this innovative system, the types of data included in BIMS, and the anticipated final product. BIMS is a secure web-based system that currently hosts over 13,000 data sources, including over 1,600 map layers, 11,000 reports and articles, and 550 Excel and Access data tables. Baseline data on natural resources in the Gulf of Mexico includes information for invertebrates, fish, reptiles, birds, and mammals, as well as habitat data. Surface



water, sediment, and biological tissue data are included to characterize the baseline chemistry and toxicity. Physical, chemical, and biological stressor data includes (but is not limited to) information on petroleum releases, non-petroleum releases, hypoxic zones, harmful algal blooms, erosion, coastal development, shoreline modification, and extreme weather conditions. BIMS is a compilation of publicly available government monitoring data, peer-reviewed literature, geospatial data, and interpreted, transcribed, and standardized data. BIMS has fully-linked and traceable information, intuitive searching and displays, and full map creation and data export functions. Even though BIMS is still under development, it is proving to be a useful tool for understanding the baseline conditions of the Gulf of Mexico prior to the Deepwater Horizon accident.

**MP077 Tracking the disappearance of oil from Gulf Coast marshes following the MC-252 Deepwater Horizon Incident** G. Harmon, Senior Project Scientist / Ecology / Cardno ENTRIX, Cardno Entrix / Senior Project Scientist / Ecology; A. Koonjebharay, Cardno Entrix. Data collected during the Summer 2010, Fall 2010, Spring 2011 and Fall 2011 at fixed sites throughout the Gulf of Mexico were used to track the disappearance of oil from Gulf coast marshes. Natural processes account for most of the oil removal although 14 of the 202 sites monitored were treated to facilitate these natural processes. These data were collected during cooperative Natural Resource Damage Assessment studies conducted by gulf state Trustees, the National Oceanic and Atmospheric Administration, and BP. Assessment sites were randomly selected from among more than two thousand Pre-Assessment survey points that were established in Summer 2010. The selected injury sites represent varying degrees of oiling and a variety of marsh habitats (herbaceous, Phragmites, back barrier, and mangroves). There are 164 sites being monitored in Louisiana, 16 sites in Alabama, and 22 sites in Mississippi. Observations were made of oiling on plant stems and on the soil surface. Observations made during the Fall 2011 assessments indicate that oil was still visible on less than 20% of the originally oiled sites. The majority of the sites where oil was visible in Fall 2011 were sites that had been initially classified as heavily oiled. In addition to the decrease in the percentage of sites that show oiling, we will also show evidence that the amount of oil on these sites has decreased significantly by Fall 2011.

**MP078 Polycyclic aromatic hydrocarbon concentrations in neuston and plankton samples from the coastal waters off Pensacola and Fort Myers, FL** I. Campbell, K. Workinger, Florida Gulf Coast University; D. Rumbold, Florida Gulf Coast University / Associate Prof of Marine Science, Florida Gulf Coast University / Professor of Marine Science; A. Loh, Florida Gulf Coast University. The 2010 British Petroleum Macondo-1 blowout released an estimated 4.93 million barrels of crude into the northern Gulf of Mexico. Despite cleanup efforts, oil slicks near the Macondo well have been reported as recently as March 2012. Polycyclic aromatic hydrocarbons (PAHs) are a major component of oil. Neuston (from surface tows) and plankton (from oblique tows beginning at mid-depth) samples were collected using a 150-mm mesh net off the coast of Fort Myers Beach and Pensacola Beach, FL. Samples were collected at three sites along these two transects from 10 km to 40 km offshore between February 2011 to March 2012. Polycyclic aromatic hydrocarbons were extracted using microwave extraction and quantified via gas chromatography mass spectrometry. Concentrations of PAHs in neuston and plankton samples ranged from less than 2 ng/g to greater than 50 ng/g. Concentrations of PAHs were lower from sampling sites within 16km of shore. Results from this study will be utilized in a comprehensive integrated study on the potential residual effects from the blowout that includes *ex situ* bioassays and chemical assays of water samples collected concurrently with these neuston/plankton samples.

**MP079 Impacts of Hurricane Isaac and Tropical Strom Lee on Mobilizing Deepwater Horizon Oil Spill Residues along Alabama's Beaches** P. Clement, Auburn University / Department of Civil Engineering; J. Hayworth, V. Mulabagal, F. Yin, G. John, Auburn University. In June, 2010, crude oil from the Deepwater Horizon (DH) incident began washing ashore along Alabama and Florida coastlines. Alabama's sandy beaches were heavily inundated by emulsified oil which survived all emergency actions during the 150 mile journey from the DH well to Alabama's shoreline. Close to the shoreline, an unknown amount of emulsified oil interacted with suspended particulates and submerged, forming relatively stable submerged oil mats (SOMs). Emerging research suggests that although the lighter petroleum components in the submerged oil could have weathered rapidly while the oil

was floating over open waters, the heavier components, such as high molecular weight polycyclic aromatic hydrocarbons (PAHs), could have resisted weathering processes. Therefore, the fate of PAHs sequestered in SOMs remains uncertain. In this study, we examine various environmental issues related to SOMs using both observational and chemical characterization data. Field data was collected immediately after several high energy weather events, which included Tropical Strom Lee and Hurricane Isaac that impacted the Gulf Shores region in September of 2011 and 2012, respectively. Our observational data show that the level of tar ball activity associated with SOMs continues to exceed the expected background level by several orders of magnitude. Our chemical characterization data show that the concentration levels of several hazardous PAHs in SOM and tar ball samples collected after Tropical Strom Lee and Hurricane Isaac are almost identical to the levels observed in the oil emulsions that arrived on Alabama's beaches back in June, 2010. These results indicate that an unknown amount of submerged oil still remain trapped near Alabama's beaches; also, several high molecular weight PAHs trapped in these submerged oil material are relatively recalcitrant. More studies are needed to better assess the potential long-term hazards posed by these partially weathered oil residues to Alabama's near-shore environments and the associated ecosystem.

**MP080 PAH concentrations and bioassays of sea-surface microlayer and subsurface water collected from the Gulf of Mexico off Pensacola and Ft. Myers, FL** A. Loh, I. Campbell, Florida Gulf Coast University; J. Guymarch, S. Le Floch, CEDRE; K. Workinger, D. Grant, P. Barreto, J. Barreto, Florida Gulf Coast University; D. Rumbold, Florida Gulf Coast University / Associate Prof of Marine Science, Florida Gulf Coast University / Professor of Marine Science. Polycyclic aromatic hydrocarbons (PAHs) are a major component of crude oil. Despite cleanup efforts, oil slicks near the 2010 British Petroleum Macondo-1 well have been reported as recently as March 2012. We will present PAH concentrations in water samples collected from the sea-surface microlayer (SSML), subsurface water (0.5m) and from mid-depth along two transects out to 40 km offshore of Pensacola and Fort Myers, Florida between February 2011 and March 2012. At each of three stations along the transects, up to 2 liters of SSML was collected using a radio-controlled autonomous sampler with a rotating Teflon drum. Subsurface water (from 0.5m and mid-depth) was collected using a peristaltic pump from the sampler or a GoFlo (closed – open – closed) bottle. All water samples were then analyzed for PAHs using the Stir Bar Sorptive Extraction (SBSE or Twister™) technology and thermal desorption gas chromatography mass spectrometry. Not surprisingly due to their ubiquitous nature, PAHs were detected in several samples. However, data have not yet been compared to the Macondo oil chromatograms and, thus, the source of the detected PAHs remains uncertain. Additionally, all collected water samples were screened for toxicity using a battery of *ex situ* bioassays consisting of bacterial luminescence (*Vibrio fischeri*), urchin development (*Lytechinus variegates*) and survival of adult copepods (*Acartia tonsa*). Several of the samples, particularly samples of SSML were found to be toxic. Results from this study will be utilized in a comprehensive integrated study on the potential residual effects from the blowout that includes chemical assays, enzyme induction assays and morphological assessment of neuston and plankton samples collected concurrently with these water samples.

**MP081 The Deepwater Horizon Oil Spill: Interlaboratory Comparison Exercises and Certification of Standard Reference Material 2779 Gulf of Mexico Crude Oil** J.R. Kucklick, National Institute of Standards and Technology (NIST) / Analytical Chemistry Division, National Institute of Standards & Technology / Hollings Marine Laboratory; M. Schantz, B. Benner, National Institute of Standards and Technology. The Deepwater Horizon (DWH) oil spill began on April 10, 2010 and continued four months releasing approximately 5 million barrels of oil that impacted about 500 miles of Gulf of Mexico shoreline. Assessing the spill's impact would require a massive effort by local, state, and federal agencies as well as private companies. A major challenge to responding to the spill was a need to understand the quality and comparability of data for oil-related compounds being generated by dozens of laboratories involved in the damage assessment. To assist with the damage assessment from the DWH spill, the National Institute of Standards and Technology (NIST) in collaboration with the National Oceanic and Atmospheric Administration (NOAA) conducted four interlaboratory measurement exercises for parent polycyclic aromatic hydrocarbons (PAHs), alkyl PAHs and oil marker compounds in sediment, mussel tissue, oil, and an exercise for PAHs in whole blood and

plasma. For each exercise, participants were requested to provide data on a reference sample, typically a Standard Reference Material (SRM) if available, and an unknown sample. This resulted in approximately 100 data sets for about 90 target compounds. The exercises provided important information on lab comparability of PAH measurements and on the scope of PAH-related compounds that can be measured by different laboratories. Data from laboratories participating in the oil exercise was also an important contribution to the certification of Standard Reference Material (SRM) 2779 Gulf of Mexico Crude Oil. This reference material greatly increases the number of values for PAH-related compounds relative to other NIST crude oil SRM. SRM 2779 has certified values for 20 individual PAHs and reference values for additional PAHs, alkyl PAH groups, hopanes and steranes.

**MP082 Sediment, Tissue, and Weathered-Oil Sample Holding Times for Oil Spill Monitoring** D. Shea, North Carolina State University / Department of Biology; W. Thorsen, K. O'Neal, P. Lazaro, North Carolina State University.

It has been over two years since the Deepwater Horizon (DWH) incident in the Gulf of Mexico where hundreds of thousands of samples were collected, but many were not analyzed within published maximum holding times (MHT) and even today there are samples of interest that have yet to be analyzed. The establishment of MHTs usually is driven by shorter-term durations that are typical of most environmental monitoring. However, the DWH incident is atypical in that the enormous number of samples made it impractical to analyze all samples of interest within published MHTs. To provide data to help assess the integrity of PAH data from samples held beyond the published MHTs, we reviewed the literature and historical data from our laboratory on the stability of PAHs and hopane in sediment, tissue and weathered oil when stored frozen at -20 C and -80 C. Analyses were conducted over an 8-year period for the following samples. 1) Weathered oil samples, oiled sediment, and oiled mussels from Prince William Sound, Alaska following the *Exxon Valdez* Oil Spill. 2) Oysters and passive sampling devices (PSDs) exposed in the laboratory to a mixture of crude oil and creosote. All samples were extracted, fractionated, and analyzed by GC/MS according to methods described previously. Our results at -20 C indicate that after 4 years, for weathered oil, losses were observed for parent naphthalene, C1-naphthalene, and parent fluorene at close to 25%, with no further loss at 8 years, and with no statistically significant loss of other PAHs or hopane. PAH decreases were somewhat greater in sediment fortified with PAH in the laboratory compared to sediment oiled in the field. Bivalves generally had less depletion of PAH compared to sediment and mostly just parent naphthalene. PSDs had somewhat greater PAH loss than sediment. At -80 C, there was a consistent, but statistically insignificant lower rate of PAH loss. These data are consistent with samples we have analyzed more recently over the past 2 years from the DWH incident. We conclude that the proper storage of oil, sediment, and tissue for at least up to 8 years at -20 C results in only small loss of the most volatile PAH and that storage at -80 C is not necessary. Furthermore, our results provide strong evidence that oil, sediment, and tissue samples collected following the DWH incident and properly stored should remain valid for at least 8 years.

**MP083 A Novel Approach for Estimating PAH Concentrations in Bioassays with Oil** C. Greer, Queen's University / Biology; P.V. Hodson, Queen's University / School of Environmental Studies; R.S. Brown, Queen's University / Department of Chemistry.

Toxicity tests often generate large numbers of samples that require chemical analysis to quantify the concentration of oil to which the organisms are exposed. Currently, the preferred approach is to quantify total polycyclic aromatic hydrocarbons (TPAH) by gas chromatography-mass spectrometry (GC-MS). However, GC-MS is very costly and time consuming, and typical budgets cannot accommodate a thorough analysis of all water samples. Fluorescence spectrophotometry is an alternative method that compares the magnitude of fluorescence of a sample to a standard curve of known concentrations of whole oil, to estimate the concentration of oil in the sample through detection of the fluorescent compounds. Although fluorescence is faster and more economical than GC-MS, it does not directly measure dissolved PAH. Our objective was to estimate TPAH by correlating GC-MS and fluorescence data. We developed a fluorescence standard curve from dilutions of the stock solution used for dosing exposure tanks and analyzed TPAH in the same stock solution by GC-MS to convert the standard curve to TPAH. Fluorescence analysis of individual toxicity test solutions provided an estimate of TPAH in test solutions, and the accuracy of these estimated PAH concentrations was assessed by a limited number of GC-MS analyses of the test solutions.

With some modifications, this strategy of combining a limited number of GC-MS analyses with more thorough fluorescence analyses provided a faster and more economical way to describe exposure conditions of toxicity tests.

**MP084 Overestimation of PAH Bioavailability When Using Passive Sampling Devices in Oil Spills** D. Shea, North Carolina State University / Department of Biology; D. Forestier, W. Thorsen, K. O'Neal, P. Lazaro, North Carolina State University.

The use of passive sampling devices (PSDs) to measure bioavailable PAH exposure resulting from spilled crude oil has been criticized due to the potential for oil to adhere to the surface of the PSD. If this oil is not completely removed prior to analysis, the PAH in the oil would be measured along with the PAH absorbed in the PSD yielding a total PAH concentration in excess of that considered dissolved or bioavailable. This would be analogous to measuring PAH in water containing oil droplets, mussels, etc. To determine whether the presence of oil adversely affects the performance of the PSD, we compared the accumulation of PAHs in four types of PSDs to that in mussels under conditions with varying amounts of fresh and weathered crude oil. The PSD types included: semi-permeable membrane devices (SPMDs), polyethylene (PE), polydimethylsiloxane (PDMS) and polyoxymethylene (POM). We found that all four types of PSDs can greatly overestimate the bioavailability of PAHs to mussels in the presence of oil, with POM having the least bias. Under the conditions used in our laboratory exposures, the physical contact of oil with the PSD increases accumulation up to 1000-fold while exposure of mussels to the same oil conditions results in only a 3-fold increase in PAH. In heavily oiled exposures, PSDs are highly enriched in total PAH compared to mussels and the relative abundance of PAHs in the PSD matches that of the source oil. Furthermore, nearly all of the PSDs that had this PAH enrichment also contained hopane – a highly water insoluble biomarker of the source oil – that should not accumulate in the PSD through the dissolved phase. Conversely, hopane was not detected in any of the PSDs that did not show this PAH enrichment. In water accommodated fraction (WAF) exposures where source oil was not present, the PSDs were not enriched in PAH compared to mussels and the relative abundance of PAHs in the PSDs matches that expected from accumulation via dissolved phase. This work demonstrates that SPMDs and other PSDs can be an inappropriate means of estimating bioavailable PAHs in the presence of crude oil and that detailed PAH analysis and the measurement of sterane and/or hopane source-oil biomarkers is critical to determining whether the PSDs provide valid data on the dissolved or bioavailable PAH in water resulting from spilled oil.

**MP085 Utilizing Comprehensive Methodologies to Examine Chemical Contaminants Including Oxygenated PAHs (OPAHs) in Gulf of Mexico Complex Mixtures** S.G. O'Connell, Oregon State University / Environmental and Molecular Toxicology; T. Haigh, Oregon State University / Environmental and Molecular Toxicology Dept; S.E. Allan, / Environmental and Molecular Toxicology, Oregon State University / Graduate Researcher; G. Wilson, Oregon State University / Environmental and Molecular Toxicology Dept; K.A. Anderson, Oregon State University / Environmental & Molecular Toxicology, Oregon State University / Dept. of Environmental & Molecular Toxicology.

Passive samplers are deployed to examine bioavailable fractions of contaminants in a variety of matrices including air, water, and sediments. New deployment devices and materials have been developed in the last decade to sample contaminants with wide ranges of physiochemical properties, including non-polar and polar organic compounds. In this research, passive sampling devices (PSDs) consisting of lipid-free polyethylene tubing were used in four locations in the Gulf of Mexico beginning in May of 2010, prior to shoreline oiling of the Deepwater Horizon Oil Spill. Further field efforts were continued in Florida, Mississippi, Alabama, and Louisiana to capture events during and after visible oil was present at a few of these locations through May, 2011. Extracts from these passive sampling devices were used to characterize PAHs spatially and temporally in previous work, but subsequent analyses of these samples have resulted in the quantitation of non-target emerging contaminants like oxygenated polycyclic aromatic hydrocarbons (OPAHs) through liquid and gas chromatography mass spectrometry (LCMS, GCMS, respectively). OPAHs are characterized in recent publications as an emerging class of contaminants with environmental concentrations and toxicities similar to PAH analogues, and by using parallel and complementary OPAH methods, we were able to target 24 OPAH compounds. Additionally, after full scan analyses of these extracts in both chromatographic systems, unknown peaks several orders of magnitude greater than some PAH or any OPAH compounds were



identified as sulfonated hydrocarbon chains. These compounds are typically used as detergent surfactants, and were identified through mass selection techniques using both LC-MS/MS quadrupole and time-of-flight (TOF) analyses. Additional screening of 1200 analytes using GC-MS Automated Mass Spectral Deconvolution and Identification System (AMDIS) revealed several other unique compounds. Current efforts are ongoing to identify as many compounds that may be toxicologically relevant in these PSD extracts from the Gulf of Mexico.

**MP086 Preparation and characterization of oil-water dispersions by pressurized flow injection (PFI) of oil into a continuous turbulent flow generator** B. Hansen, SINTEF Materials & Chemistry / Marine Environmental Technology; P. Gardinali, Florida International University / Department of Chemistry and Biochemistry; B.A. Stubblefield, Oregon State University, Oregon State University / Environmental and Molecular Toxicology; G.M. Rand, Florida International University / Ecotoxicology & Risk Assessment; O.X. Pelz, BP / Gulf Coast Restoration Organization, BP Gulf Coast Restoration Organization / GCRO, BP / GCRO; T. Nordtug, SINTEF Materials and Chemistry / Marine Environmental Technology. Preparation of oil water dispersions (OWDs) as exposure media for toxicological testing is usually accomplished by subjecting mixtures of oil and water to a defined amount of energy such as controlled agitation in an aspirator bottle or mixing by agitating the water with pumps or propellers. Most of these systems are static and the resulting oil concentration and droplet size distribution is highly dependent on the oil properties and also the geometry of the generator systems. As an alternative to these methods a system for generating defined OWDs in flow systems was developed. A recent report on a pressurized fluid injection (PFI) droplet generator (Nordtug et al, 2011) introduced a simple design to systematically deliver oil into a turbulent but controlled water stream. The method is based on continuous generation of oil dispersion with defined oil droplet size distribution and oil concentration by adjusting the oil:water ratio and the energy input (turbulence). The present study presents an inter-laboratory comparison of the capability of the PFI-generator to produce OWDs with different degrees of weathering. Droplet density, droplet size distribution and chemical analyses will be used to characterize the OWDs. The stability of the solutions will also be determined by following the formation and degradation of the OWDs by fluorescence microscopy, particle counting and 3D fluorescence spectroscopy. A protocol describing the operational conditions of the PFI-generator with respect to the creation of exposure media matching predicted or observed droplet size distributions during the DWH incident will be provided.

**MP087 Optical characterization of oil-water dispersions by fluorescence spectroscopy and microscopy: getting the answer in real time** K. Sandoval, Florida International University / Department of Chemistry & Biochemistry; I. Ley, A. Fernandez, Florida International University / Southeast Environmental Research Center (SERC); P.R. Gardinali, Florida International University / Department of Chemistry & Biochemistry and SERC. Oil is undoubtedly a complex mixture of hundreds of chemicals many of which are insoluble or slightly soluble in water. When crude oil reaches the sea surface it may form slicks that could be dispersed naturally by wave action, evaporated, dissolved, biodegraded or chemically dispersed. Droplet formation is an important factor controlling the environmental behavior of spilled oil since it's linked to processes like biodegradation, dissolution, organism uptake, and transport. Despite the fact that formation of oil-water dispersions are common during releases of crude oil and derivatives, the toxicological effects of oil in the environment are largely associated with the dissolved fractions of the oil. Due to natural turbulence from a release in a deepwater location and application of dispersants at the sea floor, droplet formation was an important process during the DWH release. This work describes a combination of fluorescence-based techniques to characterize oil water dispersions (OWDs) generated in laboratory experiments by providing droplet sizes, densities, concentrations, and bulk analysis of petroleum hydrocarbons in real time. OWDs were prepared by traditional techniques using aspirator bottles. Dispersions were created at different loading rates with different energy regimes (No-vortex, high-energy) and using chemical dispersants. A Horiba Yobin Fluoromax 4 spectrofluorometer was used to generate three dimensional excitation emission matrices (3DEEMs) directly from the samples. Two regions in the spectrum were used to assess dissolved components (Ex 280nm/Em335nm) vs. droplets (Ex345nm/Em440nm). Droplets were measured and counted by using an Olympus

IX-71 epifluorescence microscope. Unlike suspended solids or microorganisms, oil droplets fluoresced when excited with blue-violet radiation (330-385nm). The microscope was calibrated using fluorescent polystyrene beads at three sizes between 1 and 7µm. Results indicated that no-vortex WAF preparations produced stable, droplet free solutions. High-energy methods introduced large amounts of microdroplets of a wide range of sizes but the dispersions were highly dynamic and unstable. In contrast, chemically enhanced WAFs created under low energy condition produced more homogeneous and more stable OWDs suitable for toxicological testing. Filtration did drastically reduce the number of droplets from all OWDs; however, oil breakthrough was observed when the operational conditions were not carefully controlled.

**MP088 The contribution of oil droplets to the uptake and toxicity of oil dispersions to copepods** B. Hansen, SINTEF Materials & Chemistry / Marine Environmental Technology; D. Altin, BioTrix; A.J. Olsen, Norwegian University of Science and Technology; I.B. Overjordet, SINTEF – Materials & Chemistry; G. Rand, Florida International University; W. Stubblefield, Oregon State University; P. Gardinali, Florida International University; O.X. Pelz, BP / Gulf Coast Restoration Organization, BP Gulf Coast Restoration Organization / GCRO, BP / GCRO; T. Nordtug, SINTEF – Materials & Chemistry. The objective of this study was to investigate the potential contribution of oil droplets to adverse biological responses and uptake of oil components in filter-feeding boreal copepods (*Calanus finmarchicus*) exposed to naturally dispersed oil. In order to estimate the relative contribution of oil droplets versus the water soluble components (WSF), copepods were exposed to unfiltered (droplets) and filtered (WSF) dispersions at three different concentrations. Differences between the two parallel treatments in terms of body burden and acute toxicity were compared. Three different oils with different physical-chemical properties (paraffinic, naphthenic and naphthenic/waxy heavy end) were used in identical experiments. The copepods were exposed to 0.4, 2 and 10 mg oil/L nominally for 4 days, and controls were treated only with natural sea water. The concentrations used are for the lower concentrations within environmentally realistic concentrations, whereas the high concentration may be realistic in certain areas following dispersant treatment of surface spills or following a sea floor blow-out. Oil droplet concentrations and size ranges were analyzed daily through particle counting. Fluorescence microscopy was used to assess both the presence of oil droplets and algal material in the copepod's guts. While overall low copepod mortality was observed throughout, contribution of oil droplets to body burden increased with increasing dispersion concentrations. However, a large part of the oil body burden appears to be associated with the copepod surface, intestine and filtering apparatus and a substantial fraction of the ingested oil droplets was excreted through fecal pellets.

**MP089 Physical and chemical characterization of oil-water mixtures as toxicity exposure media: assessing composition, stability, and droplet formation** K. Sandoval, Florida International University / Department of Chemistry & Biochemistry; A. Fernandez, I. Ley, A. Smith, B. Echols, Florida International University / Southeast Environmental Research Center (SERC); G.M. Rand, Florida International University / Department of Earth & Environment and SERC; P.R. Gardinali, Florida International University / Department of Chemistry & Biochemistry and SERC. Assessing the exposure of biological endpoints to oil is usually accomplished by preparing oil-water mixtures of different chemical and physical compositions. Oil water dispersions (OWDs), water accommodated fractions (WAFs) and chemically enhanced water accommodated fractions (CEWAFs) are the three most common ones. Generally, water-soluble components are responsible for the bulk of oil toxicity so that WAFs are the best representation of typical exposure scenarios. Wave action and turbulent flow conditions add energy to environmental systems. The potential for oil droplet formation under these high-energy scenarios is usually assessed by OWDs prepared with different degrees of agitation. Dispersants are also employed in response to oil releases. The effects of dispersed droplets of entrained oil are evaluated from CEWAFs. All these scenarios depend on two key assumptions: WAFs have only "truly dissolved" hydrocarbons and OWDs, and CEWAFs are physically and chemically stable so that they can be used for toxicological testing. This study describes a series of physical and chemical procedures used to characterize the composition and stability of WAFs/OWDs and CEWAFs from the time of preparation through their use in exposure chambers. Epi-fluorescence microscopy was used to assess the presence or absence of oil droplets down to 1µm. Excitation/emission in the violet- blue region



permitted distinguishing entrained oil from non-fluorescent particles. Size calibration using dyed polystyrene beads allowed both average droplet sizes and droplet size distributions to be measured in each solution. Fluorescence spectroscopy was used to assess dissolved and entrained oil components using 3DEEMs. Results indicate that preparation of WAFs at low energy conditions (no-vortex) at a 1:1000 OWR produce droplet free-solutions. OWD droplet size distributions are energy, loading rate and oil-type dependent. Self-dispersions of oil are much more dynamic and unstable than CEWAFs (1:20 DOR). Inter- and intra-laboratory tests showed that low energy WAF preparations were analytically consistent. CEWAF preparations did introduce larger amounts of all oils tested in the dispersions but concentrations were more variable than for WAF preparations. Serial dilutions yielded reproducible and predictable concentrations. These findings point out the importance of conducting a comprehensive physical and chemical characterization of the exposure media in support of toxicological testing.

**MP090 Assessment of chemistry and acute toxicity of weathered MC252 oils: Are mass-based LC50s alone indicative of effect of weathering on toxicity?** L. Faksness, SINTEF / Marine Environmental Technology; B.H. Hansen, SINTEF Materials and Chemistry; D. Altin, BioTrix; T. Nordtug, SINTEF Materials and Chemistry; O. Pelz, BP GCRO. The water accommodated fractions (WAFs) of fresh (i.e. source oil) and weathered MC252 oils have been studied with special emphasis on chemistry and acute biological effects (algae growth (*Skeletonema costatum*) and copepod mortality (*Acartia tonsa*)). Low-energy WAFs were prepared following the CROSERF guidelines using an oil-to-water ratio of 1 to 10000 (100 mg oil/L water). The WAFs have been prepared from fresh and artificially weathered MC252 oil residues from 150 °C+ (representing surfacing oil) to 300°C+ (representing 3 to 5 days on sea). In addition, WAFs were generated from artificially weathered MC252 oil supplied by NOAA and two naturally weathered oil samples collected in the Gulf after the Deepwater Horizon well accident. The field samples were slick oil collected on July 19, 2010, by the USCGC Juniper and from the CTC barge. WAF is of special interest because components dissolved from an oil slick or from dispersed oil droplets are known to be bioavailable to marine organisms and therefore have a potential for causing toxic effects. On each WAF, measurements of volatile organic compounds (VOCs) and semi-volatile organic compounds (SVOCs, e.g. PAHs and total petroleum hydrocarbons) were obtained. The chemistry of the WAFs indicates that weathering preferentially removes the lighter, more volatile compounds and, in general, reduces the overall concentration of dissolved oil constituents in the WAF. The 100% WAFs were diluted and used in toxicity tests. Acute toxicity can be presented as specific toxicity, expressed from EC50 or LC50 values and normalized to mass-based concentration data, or relative toxicity, expressed as EC50 and LC50 values computed from %WAF. Relative toxicity indicated that weathering decreases toxicity since a higher %WAF was needed to produce toxic effects from weathered oils. The two methods of presenting the toxicity data show that toxicity depends on the specific composition of the WAF, and not just on the total WAF concentration or the weathering state of the oil.

MP091 Toxicity of MC252 oil to copepods and early-life stages of a sea urchin and finfish K. Workinger, Florida Gulf Coast University; D. Rumbold, Florida Gulf Coast University / Associate Prof of Marine Science, Florida Gulf Coast University / Professor of Marine Science; A. Loh, Florida Gulf Coast University. We evaluated the sensitivities of three different *ex situ* screening bioassays for detecting residual toxicity from the Deepwater Horizon blowout by conducting 24-hour static toxicity tests of chemically-enhanced (using COREXIT 9500) water accommodated fraction (CE-WAF) of artificially weathered MC252 oil (Source oil B). Preliminary assessment of the data indicate the 24-hr lethal concentration (LC50) for 50% of exposed adult copepods, *Acartia tonsa*, was much more sensitive than the 24-hr effective concentration (EC50) for abnormal development of exposed urchin (*Lytechinus variegatus*) embryos (i.e., resulting in abnormal development in 50% exposed). Toxicity tests utilizing development of redfish (*Sciaenops ocellatus*) were found to have a sensitivity similar to the urchins. In agreement with the published literature, skeletal deformations (e.g., lordosis and scoliosis) were the most obvious sub-lethal defect in the fish embryos following a 24-hr exposure (beginning within 5 hours of spawning). The 24-hr lethal concentration (LC50) for 50% of the exposed redfish eggs was 10x higher.

MP092 Effects of Deepwater Horizon oil exposures on the swimming performance and cardiac development of juvenile mahi (Coryphaena hippurus) E. Mager, University of Miami / Rosenstiel School of Marine and Atmospheric Science, University of Miami, RSMAS / Division of Marine Biology & Fisheries; A. Esbaugh, University of Miami, RSMAS / Rosenstiel School of Marine and Atmospheric Science, University of Texas – Austin, Marine Science Institute / Post-Doctoral Fellow; J. Stieglitz, R. Hoenig, D. Benetti, M. Grosell, University of Miami, RSMAS. Mahi (*Coryphaena hippurus*) were exposed to oils collected from the Gulf of Mexico following the 2010 Deepwater Horizon (DWH) spill to assess potential impacts on swimming performance. Two types of experiments were performed to examine both acute and chronic effects in juvenile mahi following a brief exposure to oil: (1) 48 h static exposures initiated with freshly fertilized eggs, followed by normal rearing for ~30–40 d under control flow-through conditions and (2) 24 h exposures to ~30–40 d old juveniles. All oil exposures were performed using dilutions of Water Accommodated Fractions (WAFs). To assess swimming performance, fish were subjected to an incremental velocity test using a miniature Blazka-style variable speed respirometer to obtain measures of critical aerobic swim speed ( $U_{crit}$ ) and aerobic scope. All fish were starved at least 24 h prior to swimming. Additionally, a subsample of larvae were collected immediately following the 48 h exposures to examine cardiac development as determined by *in vivo* microscopic analyses of cardiac edema and heart angle. The effects to swimming performance as well as cardiac development in mahi exposed during the embryonic/larval stage will be reported.

MP093 PAH metabolites in bile and enzymatic biomarkers in livers of reef fish from the northern Gulf of Mexico after the Deepwater Horizon Oil Spill L. Allen, D. Johnson, NOAA Environmental Cooperative Science Center, Florida A&M University; W. Patterson, J. Tarnecki, University of West Florida / Dept of Biology; C. Jagoe, NOAA Environmental Cooperative Science Center, Florida A&M University. The Deepwater Horizon Oil spill released almost 5 million barrels of crude oil into the Gulf of Mexico in 2010. Crude oil contains toxic components including polycyclic aromatic hydrocarbons (PAHs) that may harm aquatic organisms. Fish metabolize PAHs and excrete the metabolites via bile, where they can be detected fluorimetrically. Cytochrome p450 induction, which is involved in the biotransformation and excretion of PAHs, can be measured as activity of 7-ethoxyresorufin o-deethylase (EROD) in liver. We collected over 500 fish from multiple offshore locations in the Gulf of Mexico off Alabama and Florida in 2010–2011, during and after the oil spill, and measured bile metabolites and liver enzyme activities. Species included *Lutjanus campechanus* (red snapper), *Balistes caprisus* (grey triggerfish), *Pagrus pagrus* (red porgy), and *Seriola dumerili* (greater amberjack). Bile fluorescence was measured at excitation/emission wavelengths of 290/335, 341/383 and 380/430 nm to detect metabolites of 2, 4 and 5 ring PAHs, respectively, and standardized to billiverdin and protein concentrations. Most fish had bile fluorescence signals consistent with naphthol, indicative of oil exposure. Bile fluorescence decreased over the sampling period, suggesting that PAH exposure declined after the oil release ended. EROD activities were generally highest in fish collected near the well site, and differed among species and locations. Additional enzyme assays (for glutathione-S-transferase, a phase 2 detoxification enzyme, and superoxide dismutase, involved in antioxidant defenses) are underway to further characterize oil exposure and biochemical responses in these fish.

MP094 Impact of the Deepwater Horizon oil spill on the eastern oyster, *Crassostrea virginica* spermatozoa cellular alterations and fertilization success L. Donaghy, J. Vignier, B. Denkert, J. Devine, L. Haynes, Florida Gulf Coast University; P. Soudant, University of Brest – CNRS; F.E. Chu, Virginia Institute of Marine Science; J.M. Morris, M.W. Carney, J. Lipton, D. Cacula, Stratus Consulting, Inc.; A. Volery, Florida Gulf Coast University / Marine and Ecological Sciences, Vester Marine Field Station. In April 2010, the Deepwater Horizon (DWH) oil spill released 780,000m<sup>3</sup> of crude oil in the Gulf of Mexico, and a chemical dispersant, Corexit 9500A and 9527, was used to dissipate oil slicks at the wellhead and on the surface. The eastern oyster, *Crassostrea virginica*, is an economically and ecologically important species which propagates along the Gulf of Mexico. The oil spill occurred at the beginning of its spawning season. Exposure of gametes to toxicants in spawning animals such as *C. virginica*, potentially may lead to decreased fertilization success. Most studies use fertilization success as the assay end point. However, various defects of gametes can alter fertilization.

Using flow cytometry, we aimed at understanding the cellular modifications occurring in exposed sperm. Our results showed that oil from surface-collected DWH oil as well as dispersant impacted intracellular mechanisms. Although viability was not decreased, mitochondrial activity and associated ROS production were inhibited in a dose-dependent way. Inhibited mitochondrial metabolism would decrease the energy production needed for sperm motility. Furthermore, acrosomal integrity, which is primordial for fusion between sperm and egg, was also altered. This might also be responsible for decreased fertilization. Flow cytometry provided new insights on the intracellular damages occurring in sperm during hydrocarbons and dispersant exposures, potentially leading to unsuccessful fertilization.

**MP095 Evaluation of toxicity of Deepwater Horizon oil and dispersant on gametes of the oyster *Crassostrea virginica*: embryogenesis and larval development** J. Vignier, Florida Gulf Coast University / Marine and Ecological Sciences; B. Denkert, J. Devine, L. Donaghy, L. Haynes, Florida Gulf Coast University; P. Soudant, University of Brest – CNRS; J.M. Morris, M.W. Carney, J. Lipton, D. Cacela, Stratus Consulting, Inc.; F.E. Chu, Virginia Institute of Marine Science; A. Voley, Florida Gulf Coast University / Marine and Ecological Sciences, Vester Marine Field Station. The accidental explosion of the MC252 drilling rig off the coast of Louisiana on April 20, 2010, released 4.9 million barrels of crude oil in the Gulf of Mexico, and some 3 million liters of chemical dispersants, Corexit 9500A and 9527, were used directly at the well head and on surface to break oil slicks. This was the largest oil spill in US history after the Exxon Valdez. The eastern oyster, *Crassostrea virginica*, is an ecologically and economically important species along the Gulf of Mexico, representing about \$67 million (2009). The oil spill occurred during the spawning season of *C. virginica*; therefore the exposure of gametes to contaminant might be potentially toxic and decrease fertilization success and impact embryonic and larval development. The aim of this study was to examine the effects of acute exposure to surface-collected Deepwater Horizon oil, dispersant alone (Corexit 9500A) and dispersed oil on gametes (eggs and sperm) of oysters. Fertilization success, embryo development, larval growth and survival were assessed at different time points (1h, 24h, and 96h). Lethal and sub lethal concentration ranges were also determined. Our results showed that dispersant only and oil and dispersant combined significantly impacted fertilization rates, developmental success, viability of the larvae and growth in a dose-dependent way. Oil alone significantly decreased the fertilization success in a dose-dependent manner, and highly altered larval development and subsequent survival.

**MP096 Effect of artificially weathered oil from Deepwater Horizon oil spill and dispersants on the early life stages of oysters** J.M. Devine, J. Vignier, B. Denkert, L. Donaghy, L. Haynes, Florida Gulf Coast University; P. Soudant, University of Brest – CNRS; F.E. Chu, Virginia Institute of Marine Science; J.M. Morris, M.W. Carney, J. Lipton, D. Cacela, Stratus Consulting, Inc.; A. Voley, Florida Gulf Coast University / Marine and Ecological Sciences, Vester Marine Field Station. The estuaries and coastal areas of the Gulf of Mexico are ecologically and economically important serving the recreational and commercial needs of the population. Critical habitats such as oyster reefs in addition to serving ecological roles of biofiltration, wave mitigation and enhancement of water quality, also serve as refuge for recreationally and commercially important fishery species. In addition, oysters are also commercially important. The purpose of this study was to determine the toxicity of Deepwater Horizon oil and dispersant to eastern oysters *Crassostrea virginica*. The effects of the water accommodated fractions (WAFs) of artificially weathered source oil, Corexit9500 and oil-dispersant mixtures were examined on early life stages of *Crassostrea virginica*. Hatchery spawned oysters from an unexposed region of the Gulf of Mexico allowed for collection and exposure of oysters at each of the following life stages; gametes, embryos, D larvae, and early spat. End points such as survival, larval development, and developmental abnormality were examined. Results suggest a dose-dependent response to oil, dispersant and oil-dispersant mixtures by early life stages of oysters.

**MP097 Biological Responses of the Eastern Oyster *Crassostrea virginica* Exposed to Deepwater Horizon Oil and Dispersants via Dietary Pathways** A. Voley, Florida Gulf Coast University / Marine and Ecological Sciences, Vester Marine Field Station; L. Haynes, N. Martin, L. Castret, A. Griffith, L. Donaghy, Florida Gulf Coast University; P. Soudant, University of Brest – CNRS; F.E. Chu, Virginia Institute of Marine Science; J.M.

Morris, M.W. Carney, J. Lipton, D. Cacela, Stratus Consulting, Inc; B. Denkert, J. Devine, Florida Gulf Coast University. Following the British Petroleum oil spill in the Gulf of Mexico on April 20th, 2010; oil was found washed ashore in coastal areas in the Northern Gulf of Mexico. Oil particles can adsorb onto algae, detritus, and debris that can be ingested by filter feeding organisms such as the Eastern oyster (*Crassostrea virginica*). The benthic, sedentary nature along with the economic importance of oysters renders them particularly suited for the current study on the biological effects of oil (surface-collected Deepwater Horizon oil) that were ingested by oyster via filtration. Adult oysters were exposed to oil adsorbed onto algae cells at three different concentrations and responses of oysters followed over a period of 3 weeks. Disease prevalence and intensity of *Perkinsus marinus*, tissue alterations, immune responses and survival were examined after 2 and 3 weeks. Results suggest that survival was dose dependent and immune responses were affected in oil-exposed oysters. Results related to histopathological alterations and immune responses will be discussed.

**MP098 Signals of continued exposure to crude oil in populations of adult Gulf killifish (*Fundulus grandis*) across Barataria Bay, LA** B.D. Dubansky, Louisiana State University / Department of Biological Sciences, Louisiana State University / graduate student; A. Whitehead, University of California Davis; C.D. Rice, Clemson University / Biological Sciences; F. Galvez, Louisiana State University / Dept. of Biological Sciences. Following the Deepwater Horizon oil spill in April 2010, fish were sampled from pre-oiled sites along the coastal marsh of Louisiana, Mississippi, and Alabama to obtain baseline data on the health status of Gulf killifish (*Fundulus grandis*). The six sites were identified and sampled based on predictions of crude oil landfall, though only one site, Grand Terre Island in Louisiana, actually received significant oiling. Genomic and physiological data obtained from *in situ* sampling of killifish from these sites [Whitehead et al. 2011 (PNAS)] identified Grand Terre Island as an outlier, coincident with the oiling of this site and the greater Barataria Bay estuary. Fourteen additional sites in Barataria Bay, Louisiana receiving varying degrees of oiling were subsequently identified, and adult killifish were collected and sampled *in situ* from these sites in November and December of 2010. Using ELISA with liver microsomal fractions, we show that fish sampled from oiled sites had an increase in the expression of cytochrome P450 1A (CYP1A) protein, a sentinel biomarker of exposure to aryl hydrocarbon receptor (AhR)-active polycyclic aromatic hydrocarbons (PAHs). Moreover, the mRNA levels of CYP1A and the AhR repressor (AhRR) in livers were elevated in fish from oiled locations, which correlated with subtle changes in expression of liver CYP1A as examined by immunohistochemistry (IHC). Using IHC, we also found a high concentration of protein expression of CYP1A in the gills, head kidneys, and intestines of fish collected from heavily-oiled sites. Intestinal CYP1A protein expression was the most sensitive indicator of exposure, with increased CYP1A protein occurring generally in the more anterior regions of the intestine, indicating a gastrointestinal route of exposure to AhR-active PAHs. The data obtained from these 14 sites compared to reference sites coincides with the degree of oiling based on the Shoreline Cleanup Assessment Technique (SCAT). Additional sampling efforts in Barataria Bay in August 2011 at select sites also showed a clear signal of continuing exposure to crude oil, with Grande Terre showing a very high transcriptional profile of exposure to AhR-active PAHs.

**MP099 The effect of oil dispersants and salinity on the biodegradation of south Louisiana crude oil and impacts on Gulf killifish *Fundulus grandis*** A. Kuhl, LSU Aquaculture Research Station; J.A. Nyman, Louisiana State University / Renewable Natural Resources; C.C. Green, Louisiana State University / Renewable Natural Resources. Chemical dispersants like COREXIT are an important technology in the remediation of oil spills in the aquatic environment. Further, it has been demonstrated that environmental salinity is an important factor in the effectiveness of the dispersants on the breakdown of crude oil. Thus the purpose of this work is to investigate the effect of a dispersant on the degradation of crude oil and study the effects of varying oil degradation on acute toxicity and a sublethal physiological response in a common estuarine fish at various salinities observed in an estuarine environment. We hypothesize that biodegraded oil in the presence of dispersant will persist longer and have greater toxicity than non-dispersed oil. Laboratory mesocosms were designed with oxic water at salinities of 4, 12, or 18 g/L overlying sediment that has an oxic surface layer and an anoxic subsurface, simulating various estuarine conditions. Mesocosm water was spiked with various combinations of crude oil and of COREXIT 9500 at



the manufactures recommended dilution ratio (1:10). Treatments consist of water mixed with crude oil alone, COREXIT alone, a combined exposure to crude oil and COREXIT, and a control and allowed to biodegrade in mesocosms for 1, 4, and 16 weeks prior to harvesting water for analytical analysis and acute toxicity testing. Acute toxicity was assessed using a 96-hr static-renewal acute toxicity bioassay and juvenile common Gulf killifish (*F. grandis*). Following exposures, fish were harvested and cytosolic and nuclear protein fractions were isolated. Ethoxyresorufin-O-deethylase (EROD) and glutathione-S-transferase (GST) enzyme activity were measured and used as a biomarker of sublethal polyaromatic hydrocarbon (PAH) exposure. Results demonstrate a marked increase in PAH concentrations and resultant acute and sublethal toxicity in dispersed oil versus non-dispersed oil. Additionally, the toxicity of the dispersed oil was detectable at the greatest dilutions and persisted through 16 weeks of biodegradation. Moreover, dispersant alone was acutely toxic at the manufacturers recommended dispersant:oil ratio without activating the AhR response system. This toxicity increased at salinities outside the iso-osmotic conditions. This data indicates that dispersed oil increased the bioavailability of PAH resulting in both greater acute and sublethal toxicity.

**MP100 Acute and Chronic Toxicity of Unweathered and Weathered MC252 Oil to Mysid Shrimp (*Americamysis bahia*) and Tidewater Silversides (*Menidia beryllina*)** M.T BenKinney, Exponent; O.X. Pelz, BP / Gulf Coast Restoration Organization, BP Gulf Coast Restoration Organization / GCRO, BP / GCRO; G.M. Rand, Florida International University / Ecotoxicology & Risk Assessment. A variety of biological studies have been initiated in response to the Deepwater Horizon Mississippi Canyon 252 (MC252) spill to characterize potential effects from fresh and weathered MC252 crude oils. Laboratory tests have been conducted with mysid shrimp (*Americamysis bahia*) and tidewater silversides (*Menidia beryllina*) using a series of field-collected MC252 crude oils (MASS oil, CTC Barge oil, Juniper oil) that represent a continuum from fresh to highly weathered. Test organisms were exposed to water accommodated fractions (WAFs) of the oils prepared under slow stir non-vortex conditions for 20 hours with a 4 hour settling period before decanting. Static acute tests were conducted for 48 hours exposure in closed containers with zero headspace. Serial dilution of the freshly-prepared (100%) WAF using a standardized 1:1000 oil to water ratio was used to prepare the solutions evaluated in the static tests. Chronic tests were conducted with daily renewal of WAFs during the 21 (shrimp) or 28 (fish) day exposures. Three exposure concentrations (0.01, 0.1, and 1.0 g oil/L water) and a laboratory control were evaluated in the chronic tests. Analytical characterization of the test solutions included volatile hydrocarbons, polycyclic aromatic hydrocarbons (PAHs) and alkylated PAHs, and saturated hydrocarbons (SHC- alkanes). Results from the acute and chronic WAFs will be compared among the tests and with the parent oils. Based on the test results, the weathered oils (CTC, Juniper) were significantly less toxic than the fresher MC252 source oil (MASS oil).

**MP101 Macondo crude oil from the Deepwater Horizon oil spill disrupts specific developmental processes during zebrafish embryogenesis** Y. de Soysa, A. Ulrich, Smith College / Department of Biology; T. Friedrich, University of Massachusetts / Department of Molecular and Cellular Biology; D. Pite, Smith College / Department of Chemistry; S. Compton, D. Ok, R. Bernardos, Smith College / Department of Biology; G. Downes, University of Massachusetts / Department of Molecular and Cellular Biology; S. Hsieh, Smith College / Department of Chemistry; R. Stein, M.C. Lagdameo, Smith College / Department of Biology; K. Halvorsen, Smith College / Department of Mathematics; L. Kesich, Smith College; M. Barresi, Smith College / Department of Biology. The Deepwater Horizon disaster was the largest marine oil spill in history, and total vertical exposure of oil to the water column suggests it could impact an enormous diversity of ecosystems. Water soluble components of crude oil and specific hydrocarbons have been shown to cause defects in cardiovascular and craniofacial development in a variety of teleost species, but the developmental origins of these defects are unknown. We tested whether water accumulated fractions (WAF) of the Deepwater Horizon oil could impact specific processes throughout embryonic and larval development in zebrafish. WAF treatments caused defects in craniofacial development and circulatory function similar to previous reports, but we extend these results to show they are likely derived from an earlier defect in neural crest cell development. Moreover, we demonstrate that exposure to WAF also causes deformations in programmed cell death, locomotor behavior, sensory and motor axon

pathfinding, somitogenesis, and muscle patterning. Interestingly, the severity of cell death and muscle phenotypes decreased over several months of analysis, which was correlated with a rapid drop-off in the aromatic and alkane hydrocarbon components of the oil. Whether these teratogenic effects are unique to the oil from the Deepwater Horizon oil spill or generalizable for most crude oil types remains to be determined. This work establishes a model for further investigation into the molecular mechanisms behind crude oil mediated deformations. In addition, due to the high conservation between vertebrates, our work also provides a platform for more focused assessment of the impact that this oil spill has had on the early life stages of native fish species in the Gulf of Mexico and the Atlantic Ocean.

**MP102 Effects of crude oil, dispersants (Corexit 9500 and 9527), and mixtures on sheepshead minnows** K.J. Kroll, University of Florida / Physiological Sciences; A.C. Mehinto, University of Florida / Department of Physiological Sciences and Center for Environmental and Human Toxicology; L. Escalon, UA Army Engineer Research and Development Center; N. Garcia-Reyero, Mississippi State University / Institute for Genomics Biocomputing and Biotechnology; N.D. Denslow, University of Florida / Department of Physiological Sciences and Center for Environmental and Human Toxicology, University of Florida / Dept. of Physiological Sciences &c. On April 20, 2010 the Deepwater Horizon oil rig ruptured releasing crude oil into the Gulf of Mexico for 4 months. Oil spill volumes are estimated to be on the order of 4.9 million gallons. In order to breakup the oil, two different dispersants Corexit 9500 and 9527 were used at a ratio of 1:10- 1:50 (dispersant:oil volume) as recommended by the manufacturer. About 1.8 million gallons of the dispersants were reportedly used during the spill. Quantities of the dispersant type used during the spill have not been disclosed. Although research on the effects of crude oil has been studied, the toxicity of the dispersants and their mixtures on different life stages are very limited, especially for Corexit 9527. The objectives of this study were to investigate the effects of crude oil, the two 2 dispersants, and oil/dispersant mixtures on embryo development, homeostasis, and biomarkers of reproduction of sheepshead minnows (*Cyprinodon variegatus*). To simulate weathering, the oil, 2 dispersants, and their mixtures were mixed with artificial seawater in glass carboys for 7 days. The oil was removed using separatory funnels. The resulting water accommodated fraction (WAF) was subsampled and analyzed for PAH (polyaromatic hydrocarbons) and BTEX (benzene, toluene, xylene), and dispersant fingerprinting by GC-MS. In addition, the crude oil sample used for this study was also characterized. The WAF from the Oil/Corexit 9527 mixture contained 2-5 times higher quantities of PAH's and BTEX than the oil/Corexit 9500 mixture and it was more toxic in dose-dependent exposure studies, using doses that we expected to be below the LC<sub>50</sub>. Juvenile sheepshead minnows experienced 100% mortality at Corexit 9527 concentrations >3 ppm, and in an oil/Corexit 9527 mixture (10ppm /1 ppm). There were no mortalities in crude oil WAF.

**MP103 At what molecular weight, if ever, do petroleum hydrocarbons become non-toxic to aquatic life?** D.W. Smith, Conestoga Rovers & Associates. The aquatic toxicity and solubility of many non-aromatic petroleum hydrocarbons (PHC) are opposite functions of molecular weight and carbon number (C#). Toxicity increases with molecular weight whereas solubility decreases. According to current information, PHC toxicity increases more slowly than solubility decreases. Therefore, as molecular weight increases, a point is reached where minimally toxic concentrations exceed the solubility limit, at which point this compound and heavier compounds become non-toxic. This effect is demonstrable with acute toxicity. Acute toxicity for aliphatics above about C9 (nonane) becomes progressively harder to demonstrate without resorting to supersaturation. Almost no chronic bioassays have been conducted for aliphatic, in general, and specifically with compounds heavier than about C9. Chronic toxicity for aliphatics higher than C9 are based on extrapolation of toxicity observed for lighter compounds. These extrapolations predict that the transition point to non-toxic aliphatics occurs as low as C12 (dodecane), whereas other extrapolations suggest that it occurs at around C30. Unfortunately for assessing risk of petroleum spills, this range of uncertainty encompasses the bulk of the mass of many weathered petroleum products, such as jet fuel, diesel and heating oil, and crude oils. My talk reviews the evolving science on this issue. Current evidence suggests that the answer to the title question depends on the exposure period, the species, and the risk assessor's tolerance for uncertainty.



**MP104 Chemical Dispersants Used in the Gulf of Mexico Oil Crisis Response Are Cytotoxic to Human Skin Fibroblasts** J.T. Wise, University of Southern Maine / Wise Laboratory of Environmental and Genetic Toxicology; S.S. Wise, University of Southern Maine / Wise Laboratory of Environmental and Genetic Toxicology, Ocean Alliance; H. Xie, University of Southern Maine / Wise Laboratory of Environmental and Genetic Toxicology; J. Griffith, University of Southern Mississippi / Department of Coastal Sciences; J.P. Wise Sr, University of Southern Maine / Wise Laboratory of Environmental and Genetic Toxicology. Chemical dispersants are chemicals compounds used to aid in the cleanup of crude oil spills. They became a significant public health concern in 2010 due to the BP Deepwater Horizon Oil Crisis when millions of gallons of chemical dispersants specifically Corexit® 9527 and 9500 were used to break up the crude oil. They were applied via aerial spray and deepwater injection. Toxicity of Corexit® to humans is unknown. The primary routes of exposure to these chemical dispersants are inhalation, direct dermal contact and ingestion; therefore, the objective of this study is to determine the cytotoxicity and genotoxicity of these two dispersants (Corexit® 9500 and 9527) in human skin (BJhTERT) and lung (WTHBF-6) fibroblasts. Cells were treated with and without S9 fractions with cofactors, because fibroblast cells may not readily express P450 enzymes necessary to metabolize the chemicals. Corexit® 9500 was cytotoxic to skin cells. Specifically in skin, 50, 250, 350 and 500 ppm 9500 induced 95, 89, 52, and 3 percent relative survival, respectively. S9-mediated metabolism increased toxicity inducing 78, 84, 39 and 2 percent relative survival, respectively. Corexit® 9527 was cytotoxic to skin cells. Specifically in skin, 500, 650, 850 and 1000 ppm 9527 induced 89, 83, 74, and 51 percent relative survival, respectively. S9-mediated metabolism increased toxicity inducing 87, 77, 47 and 35 percent relative survival, respectively. Ongoing and future work will consider the genotoxic effects as well as the effects of dispersed oil.

**MP105 A Novel Derivatization-based LC-MS/MS Method for Quantitative Characterization of Naphthenic Acid Isomer-group Profiles in Soil and Sediment Samples** M. Woudneh, AXYS Analytical Services Ltd; C. Hamilton, Axys Analytical Services / Client Services, Axys Analytical Services Ltd.; G. Wang, J.P. Benskin, J.R. Cosgrove, AXYS Analytical Services Ltd. Naphthenic acids (NAs) are a complex mixture of naturally occurring acyclic carboxylic acids which are found around petroleum deposits. NAs can concentrate during the extraction of bitumen from oil sands and are among the major toxic constituents of water used during this process. Management of NA-contaminated process water poses a major challenge for ensuring environmental sustainability in Alberta's oil sands. To support environmental monitoring efforts, new tools are urgently needed for accurately and reproducibly quantifying NAs in environmental samples. In this work, we developed a novel derivatization-based LC-MS/MS method that is suitable for quantitative characterization of NA isomer-group profiles in ambient soil and sediment samples. The method utilizes liquid-solid extraction with ultrasonic agitation and cleanup using strong anion exchange (SAX) cartridges. Quantification was simplified by optimizing collision-induced dissociation within the mass spectrometer to produce approximately constant response factors among the various isomer groups. To reduce their impact on NA data, ubiquitous fatty acids were chromatographically separated from NA isomer groups. We validated the method using soil samples from northern Alberta and applied principal component analysis to NA isomer signatures to distinguish natural or industry-impacted soils from those from outside Alberta, and also from NA profiles in pristine environments (i.e. far from petroleum deposits).

**MP106 A powerful technique for the analysis of metal complexation by macro-molecules – a case study of storm event distributions** E.R. McKenzie, University of California Davis / Civil and Environmental Engineering; P.G. Green, University of California, Davis; T.M. Young, University of California Davis / Civil and Environmental Engineering. High pressure size exclusion chromatography (SEC) coupled with an online inductively coupled plasma mass spectrometer (ICP-MS) is a powerful tool to assess the size dependence of metal complexation for macro-molecules (< 300 kDa) such as natural organic matter (NOM). This system was applied in the assessment of storm event samples from four land uses: highway, urban, agricultural, and natural. Wastewater effluent and biosolids runoff were also assessed. Al was associated with large macromolecules. Absorbance ( $\lambda=254$  nm) was used to detect organic matter (OM), which was primarily detected with molecular weights 3-6 kDa; Cu, Zn, and Ni were also detected in this

same size range, indicating that they were likely complexed by the OM. Cr, Mn, Co, Ni, and Pb were commonly detected as dissolved constituents (< 100 Da). Only small shift in size associated complexations were observed during the storm. SEC – ICP-MS is a powerful tool for assessing metal complexation; SEC – IPC-MS application to storm event samples revealed both complexed metals (Cu, Zn, and Ni), as well as bioavailable metals (Cr, Mn, Co, Ni, and Pb).

**MP107 A Study on the Importance of Solid-to-Water Ratios to the Absorption and Desorption Rates of PAHs in Polyethylene Passive Samplers** O. Slosser, LMU; R.G. Adams, Loyola Marymount University. Polycyclic aromatic hydrocarbons (PAHs) along with other hydrophobic organic contaminants pose significant environmental and health risks and their quantification in surface waters is important for water quality monitoring. Polyethylene (PE) passive samplers are low-cost and allow for in situ measurement of trace organic contaminants with a simple post-extraction process. In order to estimate the dissolved chemical concentration, the rate of chemical uptake in PE must be measured; however it is impacted by temperature, PE thickness, water-boundary layer thickness, biofilm thickness, and the solid-to-water ratio. In order to estimate the in situ rate of chemical uptake, pre-added performance reference compounds (PRCs) have been used to estimate first-order desorption rates. Assuming that the rates of absorption and desorption for comparable chemicals are the same, these PRC-measured rate constants are then used to estimate the extent to which equilibrium is achieved by the chemicals of interest. In this study desorption and absorption rates for phenanthrene and pyrene will be measured in laboratory experiments in order to assess the validity of the equivalent absorption and desorption rate assumption using the precise chemical measurements afforded by synchronous fluorescence. Additionally, because the ratio of the mass of PE to water impacts the rate of chemical uptake, the rate constants in the field will vary from those measured in the lab. In order to assess the importance of this phenomenon, rate constants for sorption and desorption will be measured in laboratory experiments with varying solid-to-water ratios. Preliminary results show the rates of absorption and desorption for phenanthrene are comparable. Both desorption and absorption curves for phenanthrene in water can be fit with a first order model. Experiments with varying solid-to-water ratios for phenanthrene and pyrene are in progress. Equivalent absorption and desorption rates and corrections for varying solid-to-water ratios are needed in order to correct for disequilibrium in field-deployed passive samplers.

**MP108 Advanced oil fingerprinting using comprehensive two-dimensional gas chromatography in the Hebei Spirit oil spill case** U. Yim, Korea Ocean Research and Development Institute / Oil & POPs Research Group, Korea Ocean Research & Development Institute; S. Ha, W. Shim, Korea Ocean Research & Development Institute. After the *Hebei Spirit* oil spill (HSOS) in December 2007, mixtures of three types of Middle East crude oil (total 12,547 kl) were stranded along 375 km of coastline in Western Korea. The HSOS was the worst oil spill case in Republic of Korea, and there were many forensic issues to be solved. After oil enters into marine environments, two main factors, i.e., mixing with background hydrocarbons and oil weathering, affect its hydrocarbon composition. Similar to other oil spill incidents, there was a lack of background contamination data for the spill sites. Furthermore, the alteration of the chemical composition of the spilled oils by weathering and chemical dispersion was largely unknown. To unravel these relationships, conventional tiered fingerprint approach including gas chromatography (GC) and mass spectrometry (MS) was applied to analyze fingerprint characteristics of spilled oil. Additionally, emerging fingerprinting techniques were also employed for further detail analysis. When there exists complication in oil fingerprinting like the HSOS case, more distinctive oil fingerprinting tools are required. Comprehensive two-dimensional gas chromatography (GCxGC) has the potential to revolutionize forensic oil spill analysis. GCxGC is capable of separating an order of magnitude more compounds from complex mixtures than conventional GC. GCxGC facilitates the understanding of the sources, weathering, and toxicity of unresolved complex mixtures (UCM) hydrocarbons. In HSOS case, GCxGC was promising to comprehensively evaluate the varying degree of mixing effects of the three spill sources and the abiotic and/or biotic effects of weathering. GCxGC facilitated the initial screening of various types of hydrocarbons using group type analysis and the application of isoprenoid compounds like biphytane, methyl hopanoids and benzohopanes with the

help of enhanced separation. We found these compounds had potential to be used as conservative fingerprints in the HSOS case.

**MP109 Analysis of cyclic and linear volatile methylsiloxanes in water environment** Y. Horii, K. Minomo, M. Motegi, K. Nojiri, Center for Environmental Science in Saitama. The determination of siloxanes include cyclic and linear volatile methylsiloxanes (VMS) in environment is important for the evaluation of human and environmental risks. VMS have been widely used in consumer products because VMS have low surface tension, high thermal and chemical stabilities, and believed to be inert. However, VMS are recently identified as priority chemicals for environmental risk assessment due to their persistence in the environment and bioaccumulative potentials. Analysis of VMS in environment is very challenging due to their high volatility and potential sources of background contamination. In this study, we developed a simple method for analysis of cyclic and linear VMS in water samples by using purge and trap extraction off-lined gas chromatograph/mass spectrometer (GC/MS). In chemical analysis, firstly, 500 mL water samples was gently transferred into an 1-L glass gas washing bottle and 100 ng of  $^{13}\text{C}_5$  labeled decamethylcyclotrisiloxane (D5) in acetone was added into the sample as an internal standard. To accelerate volatilization of VMS and prevent forming water, 50 g of sodium chloride was added. The gas washing bottle was purged at the flow rate of 600 mL/min using a vacuum pump. The sample trap, several types of SEP cartridges such as active carbon and XAD2 were tested, was mounted on the outlet of the gas washing bottle. The extraction efficiency in different extraction times and water bath temperatures was evaluated. After purging the samples, the sample trap was dry-purged by pure nitrogen gas for 10 min, then target chemicals were eluted with 5 mL of dichloromethane. The eluant was gently concentrated to 1 mL. Quantification of cyclic VMS include hexamethylcyclotrisiloxane (D3) to dodecamethylcyclotrisiloxane (D6) and linear VMS include octamethylsiloxane (L3) to dodecamethylpentasiloxane (L5) was performed on a GC coupled to high resolution MS or high resolution time-of-flight MS. The presence of VMS in laboratory products and reagents, GC parts, and also ambient air, are the major difficulty in the analysis of VMS. To reduce contamination, blank levels of VMS for all products and reagents used were tested. We selected silicone free or low bleed materials to achieve high precision analysis of VMS in environmental water samples. The optimized method was applied for river water samples collected from Saitama, Japan.

**MP110 Analysis of Gasoline Using Whole-Oil GC-MS Method** M.J. Benotti, Battelle / Analytical and Environmental Chemistry; R. Lizotte, K. McNerney, J. Thorn, Battelle. Analytical methods for the analysis of petroleum products include the analysis of the more volatile components by purge-and-trap gas chromatography mass spectrometry (GC-MS), the larger molecular weight hydrocarbons by GC-MS, and the saturated hydrocarbons (SHCs) by gas chromatography flame ionization detection (GC-FID). An alternative to this approach is analysis using a "whole oil" method, in which the material is directly injected into the GC-MS or GC-FID without sample preparation or dissolution in solvent. While the approach is not suited for the analysis of crude oil or heavy distillates given the additional maintenance associated with residual deposits, it provides a unique tool for the analysis of light to medium distillates and NAPL samples. The benefit of the whole oil GC-FID approach is that it allows for analysis of C3-C44 SHCs providing a more comprehensive fingerprint compared to traditional approaches where the smaller material (

**MP111 Analysis of Pesticides and Polycyclic Aromatic Hydrocarbons in Snow Using Solid Phase Micro-Extraction** C. Walsh, Oregon State University / Chemistry; J.E. Schlau, Oregon State University / Chemistry; S. Simonich, Oregon State University / Depts. of Chemistry and Environmental & Molecular Toxicology. Atmospheric transport of persistent organic pollutants (POPs), and selective removal via snowfall, provides a mechanism for deposition of POPs to remote and/or high-elevation ecosystems, including sites which might otherwise be expected to be pristine. Prior analytical methods for measuring pesticides and PAHs in snow required large (50 kg+) samples and extensive sample preparation, which limited the practical spatial and temporal scale of field studies. An analytical method using Solid Phase Micro-Extraction-GC-MS was developed to measure a wide range of persistent organic pollutants, including organochlorine pesticides (OCPs) and polycyclic aromatic hydrocarbons (PAHs) in melted snow. Field tests on water from Dixon creek in Corvallis, Oregon and snow collected at HJ Andrews Experimental Forest in the Oregon Cascade Range showed the

detection and quantitation of these POPs at trace concentrations (ng/L) in 40 mL sample volumes.

**MP112 Application of Passive Air Samplers to Assess the Vertical Variability of Semi-Volatile Organic Contaminants in the Atmosphere of Hawaii** X. Zhang, University of Toronto Scarborough / Chemistry, University of Toronto / Environmental Sciences; Y. Lei, University of Toronto Scarborough; J. Barnes, Mauna Loa Observatory; F. Wania, University of Toronto Scarborough / Dept. of Physical & Enviro. Science, University of Toronto at Scarborough / Dept. of Physical & Enviro. Science. Many semi-volatile organic chemicals (SVOCs) such as organochlorine pesticides and polybrominated diphenyl ethers (PBDEs) have been ubiquitously detected in the global atmosphere, even in remote regions where such chemicals have never been produced or used. While many studies have focused on SVOCs transported to remote polar and highland regions, few studies have investigated SVOCs undergoing long range transport to remote tropical islands far from emission sources. The global long range atmospheric transport of SVOCs occurs mainly in the free troposphere, where little interaction with the earth surface boundary layers occurs. Investigating the occurrence of SVOCs in the free troposphere is important to understand their long range atmospheric transport. In this study, air from the free troposphere and from the marine boundary layer of the Big Island of Hawaii were sampled at Mauna Loa (19°43'53"N, 155°2'52"W, 3398 m above sea level) and at the east coast of the island (19°32'9"N, 155°34'31"W, sea level, 50 m horizontal distance from the ocean) using flow-through air samplers (FASs). FASs were operated simultaneously at the two sites from April to September, 2011. Polyurethane foam (PUF) plugs used as the sampling medium were replaced every month. Three PUF plugs were installed in each FAS and analyzed separately to assess and correct for break-through. Each month, 3100–7700 m<sup>3</sup> and 300–800 m<sup>3</sup> of air was sampled at the Mauna Loa site and at the coastal site, respectively. Among the PBDEs analyzed in the samples using an Agilent 7000A triple quadrupole GC/MS/MS system, only BDE-47 and -99 were detected frequently over the five months. The concentrations in the marine boundary layer (4.4–24 pg/m<sup>3</sup> BDE-47 and 2.4–88 pg/m<sup>3</sup> BDE-99) were higher than in the free troposphere (0.1–0.4 pg/m<sup>3</sup> BDE-47 and 0.1–0.3 pg/m<sup>3</sup> DE-99) PBDE concentrations measured at the mountain site were lower than what has been reported for other global background sites in the Arctic and on the Tibetan Plateau, suggesting that Mauna Loa may truly represent global background levels for SVOCs. Between the two FAS sampling sites, within a horizontal distance of 50 km, the elevation changes from 0 m to 4000 m above sea levels. Along this large gradient, we also deployed XAD-based passive air samplers to assess the vertical variability of SVOCs.

**MP113 Challenges in real-time detection of trace compounds in the atmospheric matrix: Quantitative detection of alkyl amines using ion chromatography** T.C. VandenBoer, M.Z. Markovic, A. Petroff, J.G. Murphy, University of Toronto / Department of Chemistry. Two methods based on ion chromatography (IC) were developed for the detection of methyl and ethyl alkyl amines (methylamine (MA), ethylamine (EA), dimethylamine (DMA), diethylamine (DEA), trimethylamine (TMA) and triethylamine (TEA)) and  $\text{NH}_3/\text{NH}_4^+$  in online atmospheric gas-particle and size-resolved particulate samples. These compounds have recently been suggested to play an important role in the formation of new respirable particles in the atmosphere, with few quantitative measurements available. The IC methods enable selective and quantitative detection of alkyl amines at ambient atmospheric concentrations (pptv and pg m<sup>-3</sup>) in samples where significant interferences can be expected from  $\text{Na}^+$  and  $\text{NH}_4^+$ . Sample pre-concentration enabled instrumental detection limits on the order of pmol (sub-ng) levels per sample, an improvement of up to 102 over current IC methods. Separation was achieved using gradient elution on Dionex CS12A and CS17 columns. The relative standard deviations in retention times during 3 week continuous (hourly) sampling campaigns ranged from 0.1 – 0.5 % and 0.2 – 5 %, respectively, across a wide dynamic range of atmospheric concentrations. Both methods have high analytical accuracy for the nitrogenous bases ranging from 9.5 – 20 % for  $\text{NH}_3$  and < 5 – 15 % for the amines. The IC methods were interfaced with an Ambient Ion Monitor (AIM), an online gas-particle collection system, and a Micro Orifice Uniform Deposit Impactor (MOUDI). In eight urban and rural MOUDI samples, the mass loading of amines consistently maximized on the stage corresponding to particles with aerodynamic diameters between 320 and 560 nm. The molar ratio of amines to ammonium ( $\text{R}_3\text{NH}^+/\text{NH}_4^+$ ) in fine aerosol ranged between



0.005 and 0.2, and maximized for the smallest particles, implicating amines as important particle precursors in the atmosphere. During AIM-IC campaigns in downtown Toronto and Egbert, ON DMA, TMA + DEA, and TEA were observed to range from below detection limit to 10 pptv in the gas phase. In the particle phase, MAH<sup>+</sup>, DMAH<sup>+</sup> and TMAH<sup>+</sup> + DEAH<sup>+</sup> were observed to range from below detection limit up to 15 ng m<sup>-3</sup>. These results highlight the need for sensitive and selective detection of methyl and ethyl amines in addition to NH<sub>3</sub> in continuous online monitoring strategies.

**MP114 Determination of Haloacetic Acids in Water by GC/ $\mu$ ECD and Strong Anion Exchange SPE** J. Stevens, D. Smith, K. Lynam, Agilent Technologies, Inc. / Chemistries and Supplies Division. The most widely used method for disinfecting water for public consumption is chlorination. During chlorination, oxidizing agents such as hypochlorite can react with any natural organic matter or bromide present in the raw water to form disinfection byproducts (DBPs). Many of these DBPs have classified as possible human carcinogens are regulated by the US Environmental Protection Agency (EPA) under Stage 1 Disinfectants/Disinfection Byproducts Rule. Haloacetic acids (HAAs) make up the second largest group of DBPs, after the trihalomethanes (THMs). There are nine HAAs (HAA9) recommended for monitoring. Five of the acids are grouped and regulated as the sum HAA5, which has an established maximum contaminant level (MCL) of 60 ng/mL. Although no current MCL has been established for the remaining acids, monitoring for all nine Haloacetic acids (HAA9) is encouraged. EPA Method 552.3 is currently used for compliance monitoring of HAA9, along with the chlorinated herbicide dalapon in drinking water. Although liquid-liquid extraction (LLE) is typically used for HAAs analysis, solid-phase extraction (SPE) offers several advantages, including high selectivity, high capacity, reduced solvent use, reduced preparation time, and reduced cost per analysis. Recent studies have shown successful utilization of SPE for HAAs extraction. Because Haloacetic acids are anionic at pH values above their pKa, a strong anion exchanger (SAX) can be used effectively to retain and preconcentrate these analytes. In this application, a silica-based quaternary ammonium strong anion exchanger was used as the SPE sorbent. A dual column GC/ $\mu$ ECD approach provided consistent and sensitive analysis for the derivatized HAAs. The detection limit for most of the HAAs was 0.05-0.05 ng/mL. Analyte recoveries at three fortifications levels (0.2-2, 1-10, 4-40 ng/mL) ranges from 82-116% with relative standard deviations (RSDs) < 3.5%.

**MP115 Determination of Pesticides and Pesticide Degradates in Filtered Water by Direct Aqueous Injection Liquid Chromatography Tandem Mass Spectrometry** M.W. Sandstrom, L.K. Kanagy, C.A. Anderson, U.S. Geological Survey / National Water Quality Laboratory; C.J. Kanagy, U.S. Geological Survey. An analytical method that utilizes liquid chromatography tandem mass spectrometry (LC/MS/MS) was developed for determination of 252 current-use pesticides and pesticide degradates in filtered water samples from streams and ground water. The pesticides represent a broad range of chemical classes including acetanilide and triazine herbicides, fungicides and organophosphate insecticides, and were selected based on criteria such as mass applied to agricultural and urban land, probability of occurrence in streams and ground water, and toxicity to humans or aquatic organisms. One unique aspect of the method is that it includes a large number (136) of pesticide degradates. The method uses direct injection of a filtered 100- $\mu$ L water sample into the LC/MS/MS; no other sample preparation is necessary. During analysis, two injections are used, one in electrospray ionization (ESI) positive mode and one in ESI negative mode, using dynamic multiple reaction monitoring (MRM) conditions, with two MRM transitions for each analyte. The LC and MS/MS instrument parameters were optimized to select conditions that provided the highest sensitivity for the majority of analytes. Recoveries of most of the analytes were from 80 to 120 percent, with standard deviations less than 20 percent over the concentration range of 10 to 5000 ng/L in the water matrices tested (reagent water, 2 streams and a ground water). Method detection limits (MDLs) for most analytes were from 2-50 ng/L in ESI positive mode, while MDLs were about an order of magnitude higher for analytes analyzed by ESI negative mode. Interferences in some stream samples caused low recovery of an internal standard that resulted in high recoveries of some analytes, indicating that careful monitoring of internal standard responses and selection of alternate internal standards is a requirement for accurate quantification. The LC/MS/MS method minimizes sample preparation while increasing sensitivity and accuracy, and is suitable for quantification of a broad range of important

pesticides and pesticide degradates at concentration levels relevant for environmental monitoring studies.

**MP116 Determination of Pharmaceutical Compounds in Great Lakes Top Predator Fish** X. Xia, B. Crimmins, P. Hopke, Clarkson University; J. Pagano, State University of New York at Oswego; M. Milligan, State University of New York at Fredonia; T. Holsen, Clarkson University. The Great Lakes Fish Monitoring Program (GLFMP), administered by the U.S. EPA Great Lakes National Program Office (GLNPO), aims to monitor temporal trends of emerging contaminants in the Great Lakes using top predator fish as biomonitors. Recent work has focused on identifying and quantifying emerging contaminants including pharmaceuticals in lake trout due to their toxicity and potential chronic effects on the aquatic environment. In this study, an ultra-high pressure liquid chromatography-Xevo G2 Qtof mass spectrometry (UPLC-HRMS) method was developed. Reversed-phase separation of pharmaceutical target compounds was achieved using an ACQUITY UPLC<sup>®</sup>HSS T3 column, and aqueous 0.1% (V/V) formic acid and methanol as mobile phases. Sample preparation procedure and analysis method will be discussed. The analytical results including detection limits of different target compounds will be presented.

**MP117 Development and Validation of Complementary LCMS and GCMS Methods to Examine Oxygenated-PAHs (OPAHs) in Environmental Mixtures** S.G. O'Connell, Oregon State University / Environmental and Molecular Toxicology; T. Haigh, G. Wilson, Oregon State University / Environmental and Molecular Toxicology Dept; K.A. Anderson, Oregon State University / Environmental & Molecular Toxicology, Oregon State University / Dept. of Environmental & Molecular Toxicology. Despite the reports of oxygenated polycyclic aromatic hydrocarbons (OPAHs) in publications for over three decades, quantitative analyses of these compounds have only started to make significant advances within the last few years. OPAHs, alternatively labeled oxy-PAHs, have one or more oxygen attached to the aromatic ring structure, and a few of these compounds have been reported as being persistent in the environment, and potentially more toxic than their PAH analogues. OPAHs may be formed from petrogenic or pyrogenic sources, but also through chemical oxidation, photo-oxidation, or biological transformation of un-substituted PAHs. In previous studies, both gas chromatograph (GC) and liquid chromatograph (LC) mass spectroscopy (MS) methods have successfully quantitated OPAHs in environmental samples, but methods often report only a handful of compounds, thus limiting environmental investigations. Using parallel and complementary methods of LC/APCI/MS and GC/EI/MS to analyze a suite of OPAH compounds has not been reported in any literature sources to our knowledge. Utilizing both chromatographic methods provides the identification and quantitation of 24 target OPAHs (log K<sub>ow</sub> 0.2-4.81), with 19 compounds shared between methods, and 3 quantitatively determined by GCMS (Xanthone, Chromone, and Perinaphthenone), and 2 by LCMS (1,6-Benzo[a]pyrenedione, and 7,8-Benzo[a]pyrenedione) in addition to labelled and deuterated standards. All 22 OPAHs in the GCMS method were within 15% accuracy, and had less than 15% relative standard deviations (RSDs), while the LCMS quantitated 19 of 21 non-labelled compounds within 20% accuracy, and less than 15% for RSDs using calibration standards (n=6). Instrumental limits of detection ranged from 0.006-0.528  $\mu$ g/mL using the GCMS, and only 0.010-0.021  $\mu$ g/mL on the LCMS. After method validation, both methods were used to examine OPAHs in complex mixtures from the Portland Harbor Superfund Site, OR. Using passive samplers deployed at this Superfund, several semi-polar organic compounds were identified in the complex extract mixture, including OPAHs. Current work will quantify any additional OPAHs present in the extracts using both complementary methods for a full analysis of 24 OPAH compounds.

**MP118 Development of a Sediment Collection Protocol and Sampling Device for Incremental Sampling in a Mid-Atlantic Tidal Creek and Marsh** J. Barber, U.S. Environmental Protection Agency; M. Ciarlo, EA Engineering, Science, and Technology, Inc., EA Engineering, Science, and Technology; K. Rigney, D. Straume, EA Engineering, Science, and Technology, Inc.. Incremental sampling of sediments presents unique challenges in estuarine environments where a broad range of water depths, sediment types, and habitats may be encountered. An incremental sampling protocol and specialized sampling equipment were developed as part of investigation of sediments in Darby Creek, a tidal estuary located near Philadelphia, PA. The protocol targeted 50 increments of the top 3 inches of sediment in



each of 35 decision units. Decision units covered approximately 600 acres of wetland habitat which included deep open water, shallow tidal channels, high marsh, and low marsh. Daily tidal range spanned differences of up to 6 feet. Sampling was conducted from boats, canoes, and on foot. To achieve recovery of target depth and volume for each increment in a variety of habitats, sediment sampling devices were developed that consisted of 1 inch diameter cellulose acetate butyrate tubing temporarily affixed to a valve and extendable handle. A variety of valves were used to enhance recovery in different environments. Samples were extracted directly into sample containers using a plunger. In deep water environments, a box corer was used to retrieve large sediment cores which were subsampled using the device. Sampling protocols and devices were used successfully in the field with rapid rates of recovery. The field effort provided a number of lessons learned regarding incremental sampling in estuarine environments.

**MP119 Environmental Fate and Distribution of Lipitor** P.J. Pagnozzi, Rensselaer Polytechnic Institute / Civil and Environmental Engineering; M.E. Barnes, Rensselaer Polytechnic Institute; M. Nyman, Rensselaer Polytechnic Institute / Dept. of Civil and Environmental Engineering. The statin class of cholesterol lowering drugs was introduced in the 1980s and has become the most prescribed class of drugs worldwide. Lipitor (atorvastatin) was the most widely prescribed drug in the U.S. from 2000 through 2011. Given the quantity of the chemical released into the waste stream there is relatively little data on the fate and distribution of atorvastatin in the environment. Furthermore, there is little physiochemical information available on the selected drug in conventional databases. The focus of this study is to develop a relevant database which will facilitate modeling of atorvastatin transport and evaluation of groundwater contamination. The three areas under study include: (1) determination of calculated and measured physiochemical parameters including octanol-water partitioning coefficient and Henry's Law constant; (2) evaluation of the stability of atorvastatin under typical environmental conditions over time; and (3) sediment sorption studies to develop isotherm models. For example,  $\log K_{OW}$  was estimated to be 3.71 by Hammett's fragment method indicating moderate hydrophobicity. Experimental values will be obtained by high performance liquid chromatography (HPLC) and will be compared to the estimated values. Environmental stability was assessed on a monthly basis for 12 months by ultraviolet (UV) spectrophotometry at 241.2 nm under varying conditions. Stock solutions of 1 – 20 mg/L of atorvastatin in methanol or water were stored either in refrigerator or at room temperature in both light-shielded and light-exposed vessels. UV absorption was plotted versus concentration and time. At four months no significant change was found in the slope of the line for any of the six subgroups indicating stability under general conditions. Sediment-sorption studies were initiated including a 4-week equilibration study and 1- and 2-week isotherm studies to ascertain the sorption characteristics of atorvastatin (Lipitor) in the natural environment. High performance liquid chromatography (HPLC) using a 68:32 (v/v) methanol:water mobile phase yielded a retention time of 3.2 minutes. The 1-week sorption results indicate that atorvastatin is not readily sorbed to a silt-clay (e.g.,  $K_F=0.019 \text{ mg}^{-1} \cdot \text{NL} \cdot \text{g}^{-1}$ ;  $N=0.955$ ;  $r^2=0.9135$ ) sediment collected from Lake Macatawa (Holland, MI). The cumulative results of these studies will be utilized to guide the development of future research on fate and transport of these drugs in the environment.

**MP120 How Low Can You Go With High Volume Sampling?** C. Julias, CDM, CDM Smith / Chemical Engineer, CDM / Chemical Engineer; S. Kirchner, G. Molnar, CDM Smith; V. Macwan, CDM. Pesticides, dioxins/furans, polychlorinated biphenyls (PCBs), and polycyclic aromatic hydrocarbons (PAHs) are often reported as non-detects in aqueous matrices due to high reporting limits when routine analytical methods are used. That offers little insight into the actual concentrations. Achieving lower reporting limits are often desired, especially when the data are used for risk assessments. An approach is needed to obtain much lower reporting limits to determine the concentration of these compounds. To achieve such low reporting limits, a high-volume sampling (HVS) method has been utilized. The HVS technique is a method in which high volumes of water are passed through a filter containing specially packed extraction material. The filter is then analyzed to eliminate interferences associated with an aqueous matrix, thereby reducing the reporting limit. An isotope dilution technique has been successfully applied to determine PAHs in aqueous and suspended matrices and has been evaluated as an option for pesticides analysis in water. These methods and adaptations of EPA Method 1613B (dioxins/furans) and 1668A (PCB

congeners) have been applied to supplement the HVS technique to achieve the lower detection limits sometimes needed for aqueous samples.

**MP121 Identification of analyte selective passive sampling phases using Hansen Solubility Parameters** C. Hastie, C. Sparham, R. van Egmond, Unilever; T. Gouin, Unilever / Safety and Environmental Assurance Centre. Passive samplers are increasingly being used as a tool for monitoring exposure to hydrophobic organic chemicals. Recent advances have focused on expanding the use of passive samplers towards other classes of organic chemicals, such as polar, permanently charged, and ionisable organics, which are properties of chemicals typical of those used as active pharmaceutical ingredients and in personal care products. One of the challenges in developing a passive sampler for chemicals with polar or charged functionality is in selecting an optimal solid phase. Here we present the use of a screening tool based on calculations of Hansen solubility parameters for a wide range of common plastics and pollutants of environmental relevance. Results suggest that non-polar organic chemicals with  $\log K_{OW} > 5.5$  have the potential to have significant sorption capacity to plastic polymers commonly used as passive samplers, such as polyethylene and PDMS. These observations are supported by the use of Hansen solubility parameters, which indicate that non-polar organic chemicals have a greater potential for dissolution in a material like polyethylene, than do polar organic chemicals. Surface interactions for polyethylene and PDMS are characterised by van der Waals interactions, which help explain the strong dissolution capacity of these polymers for non-polar organic chemicals. Plastic polymers with hydrogen-bonding capacity and polar functional groups, however, have lower sorption capacity for non-polar organics, but may represent a suitable material for polar and ionisable organic chemicals. In this study we have assessed the applicability of using Hansen Solubility Parameters as a screening tool for optimizing the selection of polymeric materials by comparing solubility parameters calculated for analytes sampled from a sewage treatment works with those of a range of polymeric receiving phases.

**MP122 Investigating sediment heterogeneity and biogeochemical processes using two-dimensional, high-resolution passive sampler techniques** P. Teasdale, D. Welsh, W. Bennett, Griffith University / Environmental Futures Centre; A. Pages, Curtin University / Chemistry Department; J. Panther, Griffith University / Environmental Futures Centre. Trace elements in three-dimensional sediments have primarily been studied and managed using one-dimensional (1D) data. This approach has not adequately considered sediment heterogeneity, despite conceptual models of sediment heterogeneity being known since 1982. Several *in situ* measurement techniques allow trace substances to be determined in two-dimensions (2D) and at high-resolution (mm), allowing effective characterization of sediment heterogeneity and better understanding of sediment biogeochemical processes. The diffusive equilibration in a thin film (DET) technique and the diffusive gradients in a thin film (DGT) technique allow 2D distributions of several trace elements in sediment, measuring sediment pore water concentrations or resupply processes. Inexpensive colorimetric techniques that utilise computer imaging density measurements to quantify the DET and DGT samplers have been developed for iron(II), manganese(II), sulfide, phosphate, nitrite and alkalinity. 2D distributions from heterogeneous sediment microcosm or field deployments have provided fascinating new perspectives on sediment biogeochemical processes involving these elements/parameters. The capability to make measurements of two or more analytes at the same location overcomes many of the confounding issues of heterogeneity. Some data have strongly reinforced the need for 2D measurements. Complex mosaic distributions of heterogeneous iron(II) and sulfide, representing sub-oxic and anoxic biogeochemical zones, in coastal sediments would not be able to be determined using conventional sediment porewater sampling methods. 2D measurements of iron(II) and phosphate have also provided new perspectives on the major mechanisms of phosphate mobilisation. Examples are provided from several other studies of sediment biogeochemical processes. These techniques will play a vital role in improving our understanding of sediment processes and facilitating interpretation of measurements using conventional methods.

**MP124 Passive sampling devices (PSDs) and GC-MS screening tools to assess a suite of over 1,200 contaminants in agricultural areas in Western Africa** K.A. Hobbie, Oregon State University / Environmental Molecular and Toxicology, Oregon State University / Department of Environmental & Molecular Toxicology, Oregon State University; M.A. McCartney, T.A.

Haigh, G.R. Wilson, Oregon State University / Department of Environmental & Molecular Toxicology; A. Ndiaye, D. Seck, CERES-Locustox; P. Jepson, Oregon State University; K.A. Anderson, Oregon State University / Environmental & Molecular Toxicology, Oregon State University / Dept. of Environmental & Molecular Toxicology. Organizations and developing countries that attempt to assess pesticides and other environmental contaminants face many challenges of how to measure them, such as, the remoteness of sites and subsequently the difficulty of transportation and stability of chemicals in samples from these sites. The Sahel region of Western Africa has many highly engineered agriculture-based irrigated systems that generally have three cropping cycles per year. The need to assess changes in contaminants at these sites requires tools that can characterize contaminants at high spatial and temporal resolution. Modification of farming pest management practices face the challenge of how to measure success; how to assess the changes in bioavailability of contaminants. They need quantitative tools that can characterize contaminants and predict their risk to local organisms and humans. To address these issues we have further developed passive sampling devices (PSDs) that can sequester thousands of bioavailable chemicals. These devices can help organizations and developing countries to evaluate changes in pest management that may produce or release chemicals. PSDs can help organizers characterize exposure more accurately. We are using PSDs to capture relevant non-polar and semi-polar pesticides and other chemicals of interest at multiple sites in Western Africa. PSDs do not require power, transport easily and are easily extracted with organic solvent. We deployed PSDs in irrigation canals at seven agricultural sites in Senegal, Mali, and Mauritania agricultural stations. We were able to screen the PSD extracts for over 1,200 potential contaminants with gas chromatography mass spectrometry through the use of libraries we have further developed based on software, including automated compiled mass spectral de-convolution and identification system libraries that contain numerous classes of chemicals of concern (pesticides, PCBs, PAHs, pharmaceuticals, industrially related chemicals, etc.). The results for all sites combined showed positive hits of six PCB congeners, 11 pesticides, 21 PAHs and two legacy pesticides including ethiolate, p,p' DDD and its breakdown products. Furthermore the compound pentachloroanisole a breakdown product of pentachlorophenol was detected in Mauritania. PSDs coupled with GC-MS screening tools offer a powerful technology to identify persistent organic pollutants present at agricultural sites in three countries in the Sahel region of Western Africa.

**MP125 Photochemical formation of hydroxyl radical from dissolved organic matter** C. Glover, University of Colorado Boulder; F. Rosario-Ortiz, University of Colorado. The photochemical formation of the hydroxyl radical (HO•) from the photolysis of organic matter is of interest as this radical influence the fate of organic compounds in water and also the formation of low molecular weight compounds within the water column. The generally accepted pathway for the formation of HO includes the formation of an excited triplet state within organic matter (OM), followed by the oxidation of water. The quantum yield for the formation of HO from OM have been measured for a number of OM samples from different sources, including isolates, surface waters and ocean waters. The values for the quantum yields vary over close to an order of magnitude and are expected to be correlated to properties of OM. For example, the quantum yields measured for ocean waters decreased significantly as the distance from shore increases. In addition, the impact of halides (I<sup>-</sup>, Br<sup>-</sup>, and Cl<sup>-</sup>) as quenchers of the photoproduction of HO from OM was examined. Halides are known to quench excited triplet states, however no studies have characterized the impact on the formation of HO from OM. Initial results indicate the concentrations of chloride of up to 3 mM significantly reduce the formation of HO using OM isolates.

**MP126 Rapid on-site screening of oil contamination at a coarse-sediment shoreline** M. Kim, J. An, Korea Ocean Research and Development Institute / Oil & POPs Research Group; J. Won, Korea Marine Environment Management Corporation / Climate & Marine Environment Team; H. Choi, Korea Ocean Research and Development Institute / Ocean Data and Information Unit; U. Yim, Korea Ocean Research and Development Institute / Oil & POPs Research Group, Korea Ocean Research & Development Institute; C. Joo, E. Kim, B. Kim, W. Shim, Korea Ocean Research and Development Institute / Oil & POPs Research Group. Oil contamination level and extent at coarse-sediment shorelines, such as sand beach, could not be clearly characterized by conventional sediment analysis methods because quantification of oil in sediment is highly dependent on grain size and weight of sediment itself. We found that, as an alternative, oil contamination

at a sandy shoreline can be better evaluated by measuring the oil content of pore water than by measuring oil concentrations in sediments. Pore water can reflect sediment contamination and the quantification of oil in pore water is not dependent on the grain size. Pore water can be analyzed using a conventional gas chromatographic (GC) method for oil quantification. However, this analytical method is often impractical as a rapid screening tool at oil spill sites, considering the analysis time, cost, and the number of samples to be processed. In this presentation, a novel fluorometric on-site analysis method for oil in pore water is introduced and applied to the Hebei Spirit oil spill case, one of the world's largest oil spill incident in recent years. The comprehensive contamination level and temporal variation of dissolved/dispersed oil in pore water was investigated and the result was illustrated by means of GIS mapping techniques. The fluorescence detection method was also validated and compared with the conventional laboratory technique of total petroleum hydrocarbon analysis using gas chromatography. The fluorescence method for detection of oil was capable of generating data comparable to that of GC, but more rapidly and cost-effectively.

**MP127 Screening of novel halogenated aromatic hydrocarbons in environmental samples by GC coupled to high resolution TOFMS** Y. Horii, K. Nojiri, Center for Environmental Science in Saitama; N. Yamashita, National Institute for Advanced Industrial Science and Technology; K. Kannan, State University of New York; T. Ohura, Meijo University; Y. Miyake, University of Shizuoka. Recent studies reported several chlorinated or brominated PAHs (Cl-/Br-PAHs) induced AhR-mediated activity, and the relative potencies of lower-molecular-weight Cl-PAHs, such as chlorophenanthrene and chlorofluoranthene, tended to increase with increasing chlorination of the compounds. Therefore, determination of Cl-/Br-PAHs include highly substituted Cl-/Br-PAHs in environment is important. In previous studies, we have investigated occurrence of Cl-/Br-PAHs in air, sediment, soil, and waste incineration samples, and low halogenated Cl-/Br-PAHs were found to be widespread environmental contaminants. GC/MS analyses based on monitoring of molecular ions (M, M+2, or M+4) indicated the presence of some highly substituted ClPAHs in the samples, although the analytical standard was not available. In this study, screening of novel halogenated aromatic hydrocarbons in environmental samples was performed by using a GC coupled to high resolution time-of-flight MS (HR-TOFMS), which has a very narrow mass window (e.g. 0.02 Da), accurate mass measurements for the full mass range (*m/z* 60–800). The extracts of selected samples include waste incineration flue gas and fly ash, surface sediment, and biota were prepared, then were purified and fractionated using silica-gel and active carbon columns for the analyses. An aliquot of the eluant (dioxin fraction), was loaded to GC/HR-TOFMS operated in electron impact. In the preliminary result, about 40 congeners of Cl-/Br-PAHs having analytical standards were detected from fly ash samples. The calculation of the elemental composition for the detected peaks was performed in accurate mass. Highly chlorinated PAHs (e.g. C<sub>16</sub>H<sub>5</sub>Cl<sub>3</sub>) and ClBr-PAHs (e.g. C<sub>16</sub>H<sub>6</sub>Cl<sub>3</sub>Br and C<sub>16</sub>H<sub>7</sub>Cl<sub>2</sub>Br) were identified with high probability. Isotope patterns of the compounds in the samples were compared with theoretical isotope patterns for halogenated PAHs. This technique provides exhaustive analysis and powerful identification for the unknown and unconfirmed halogenated aromatic hydrocarbon congeners in environmental samples.

**MP128 Sediment Accretion Study of a Southern California Tidal Salt Marsh** G.C Weber, P. Krause, A. Beach, ERM. Historically, tidal salt marshes were plentiful along the Southern California coastline. Only a fraction of these remain today, owing to intentional filling and habitat degradation. Those that remain are primarily surrounded by developed land-uses. Consequently, the hydrodynamics and sediment transport properties of these marshes can be highly disturbed. Additionally, the drainages that feed these marshes are frequently channelized, and flow through urban and/or agricultural areas. Urban and natural discharges to the marsh may carry heavy loads of sedimentary material into the marshes which historically would not have been introduced under natural conditions. Factors such as these likely have impacted the sediment accretion rates in affected salt marshes. However, studies investigating sediment accretion rates in Southern California salt marshes are limited. The sediment accretion rate in Carpinteria Salt Marsh, located along the Pacific coast between Santa Barbara and Ventura, CA was measured using radiological techniques. Sediment core samples from several locations within the marsh were gathered using a hand driven core sampler. Cores were segmented in the field, and

then analyzed for the radioisotopes  $^{210}\text{Pb}$  and  $^{137}\text{Cs}$  in the lab. Using the analytical results, sediment accretion rates were extrapolated from measurements of  $^{210}\text{Pb}$  activity data. Similar results of  $^{137}\text{Cs}$  activity were used as a secondary means of estimating sediment accretion rates through extrapolation, by determining of the 1954 horizon and 1963 maximum in the cores. Sediment accretion rates were found to vary little across the 230 acre marsh with ranges measured between 0.1 to 0.7 cm/yr. Considering that there are limited accretion data available for Southern California salt marshes, rates measured in this study may provide a rough approximation of accretion levels in similar marshes located in Southern California.

**MP129 Selecting Surrogates for an Alkylphenol Ethoxylate Analytical Method in Sewage and Soil Matrices** L. Zintek, US EPA; C.M. Acheson, U.S. Environmental Protection Agency / National Risk Management Research Laboratory, US EPA; M. Mills, US EPA. Alkylphenol ethoxylates (APEs) are nonionic surfactants commonly used in industrial detergents. These products contain complex mixtures of branched and linear chains. APEs and their degradation products, alkylphenols, are highly toxic to aquatic organisms, potentially estrogenic, persistent in many environments, and commonly found in wastewater matrices. More information is needed to characterize environmental concentrations and the fate of these compounds. Methods have been developed for qualitative and quantitative determination of alkylphenols, APEs, and Bisphenol A in sewage sludges, biosolids, and soils. These methods utilize SPE extraction followed by UPLC/MS/MS analysis to achieve quantitation levels less than ng/kg. Quality control measures are an important part of demonstrating method performance during development and in routine measurements. One important quality control measure is surrogate recovery. Ideally, recoveries of target analytes and surrogates are strongly correlated. For these analytes, selecting surrogates is challenging due to limited off the shelf options and the costs of custom synthesis. Observed recoveries for commonly selected surrogates such as *n*-nonylphenol are not representative of many nonylphenol analytes particularly in important matrices such as sewage sludges and biosolids. Recoveries for a variety of surrogates and matrices will be discussed.

**MP130 Simultaneous determination of four metabolites of benzene and toluene in urine by liquid chromatography/tandem mass spectrometry** R. Fan, South China Normal University / Key Laboratory of Ecology and Environmental Science in Guangdong Higher Education, School of Life Science; D. Wang, J. She, California Department of Public Health / Environmental Health Laboratory Branch. This study developed a method to simultaneously determine *trans*, *trans*-muconic acid (*t*, *t*-MA), 1, 2-dihydroxybenzene (or catechol, abbreviation as 1, 2-DB), *S*-phenylmercapturic acid (*S*-PMA) and *S*-benzylmercapturic acid (*S*-BMA) in human urine. Samples were prepared through solid phase extraction (SPE) and analyzed by ultra-high performance liquid chromatography/ tandem mass spectrometer (UHPLC/MS/MS) in negative electrospray ionization mode. The method was fully validated through the studies of precision, accuracy, matrix effects, detection limit, linear range, stability and real urine sample tests. Calibration curves of all target analytes showed favorable linearity within the wide concentration range of 0.2–4,000  $\mu\text{g/L}$ . The detection limits (LODs) in 10 times diluted pooled urine ranged from 0.08  $\mu\text{g/L}$  to 7.8  $\mu\text{g/L}$ , which were several times lower than those reported in the literatures. The method showed satisfactory accuracy and precision after we measured varied levels of the target analytes spiked in diluted pooled urine. With an exception for the low spiked QC level of 1, 2-DB (73.1% recoveries), recoveries were in the range of 100  $\pm$  15% with a variation coefficient of less than 15%. Target analytes were stable in stock solutions and spiked urine samples. Twenty nine real urine samples were analyzed for all target analytes. With the exception of *S*-PMA, other three compounds could be detected and quantified. *S*-BMA has significant correlation with *t*, *t*-MA ( $r=0.656$ ,  $p<0.01$ ) and the total concentration of benzene and toluene metabolites ( $r=0.692$ ,  $p<0.01$ ). Due to *S*-BMA's prevalent existence and its positive association with *t*, *t*-MA and total concentration of benzene and toluene metabolites in urine samples of non-occupational exposure population, it would be the prior biomarkers with enough sensitivity and specificity to assess low level benzene and toluene exposure.

**MP131 Study of partitioning behavior of common fossil fuel derived fuels in water via stir bar sorptive extraction and gas chromatography/mass spectrometry** R. McCreary, University of Texas at El Paso / Department of Chemistry. Consequences resulting from accidental release of

common fossil fuel derived fuels (CFFDFs) into aquatic environments reach much deeper than just the immiscible layer of the fuel that lingers in the water. Studies have shown that there are constituents of these fuels that accommodate themselves in the aquatic environment, known as the water accommodated fractions (WAFs), which can induce adverse health effects depending on the constituents of the original fuel and their concentrations; thus, there is a great need to develop fast and sensitive methods to analyze WAF in the environment. In this study, the WAF will be extracted using an optimized stir bar sorptive extraction (SBSE) technique followed by gas chromatography/ mass spectrometry (GC/MS). By using this efficient, easy, and environmentally conscious technique, WAFs and the partitioning behavior of various CFFDFs—biodiesel, diesel, gasoline, jetfuel—will be profiled. By studying the partitioning behavior of various types of fuels in fresh water and sea water, impact of CFFDF on the aquatic environment can be more accurately accessed.

**MP132 The Analysis of Antidepressants in Aqueous Samples using Liquid Chromatography-Tandem Mass Spectrometry** K.J. Chodara, The Pennsylvania State University / Department of Forensic Science, The Pennsylvania State University; D. Sykes, M. Gettle, A. Zimmermann, The Pennsylvania State University / Department of Forensic Science. Antidepressants are psychiatric medications that are taken with the intent to alleviate mood disorders. These drugs and their metabolites enter the environment as a byproduct of use, and may pose a danger to human health and that of wildlife. In the USA, there are few environmental regulations concerning the discharge, fate and transport of such pharmaceuticals. Unfortunately, because most antidepressants exhibit high stability, they pass unchanged through the wastewater treatment process and remain biochemically active. An important first step towards developing proper regulation is the creation of a selective and sensitive detection method in the relevant matrices. This presentation will discuss the methodology developed for the detection and identification of a variety of commonly used antidepressant drugs and their metabolites. High Pressure Liquid Chromatography (HPLC) on fused core silica phases is used for the separation of analytes, and a triple-quadrupole mass spectrometer is employed using both Scheduled Multiple Reaction Monitoring (MRM) and Information Dependent Acquisition (IDA) detection methods with electrospray ionization (ESI). Sample preparation is performed in accord with EPA method 1694, using solid-phase extraction with an Oasis HLB cartridge suitable for acidic, basic, and neutral compounds. Previous studies have examined the utility of optimizing parameters such as sample matrix and SPE cartridge, the viability of isotope dilution for quantification, and the use of Ultra-High Pressure Liquid Chromatography grade columns (less than 2  $\mu\text{m}$  particle size) in terms of antidepressant detection. Little research has been done focusing on optimizing resolution by altering the composition of the columns. This study aims to examine column compositions including C8, C18, phenyl-hexyl, and amide to determine how chemical interactions between analytes and the column influence the separation and analysis of antidepressant compounds. Parameters such as efficiency, asymmetry, and selectivity will be considered to optimize the method. Internal standards will provide quantification and waste water samples will be used for validation.

**MP133 The Impact of Sediment Sampling on Environmental Quality Standards** N. Mokwe Ozonzeadi, Blackhealth / Life Sciences, Blackhealth; S. McEldowney, University Of Westminster; I. Foster, University Of Northampton / School Of Science And Technology; E. Valsami-Jones, University Of Birmingham / School Of Geography, Earth And Environmental Sciences, Edgbaston. The impact of contaminated sediment on water quality has gained significant interest in recent years. The onset of the European Water Framework Directive is a key driving factor for the better management of water bodies and contaminated sediment. At present in the UK, there are no quantifiable environmental quality standards (EQSs) for suspended or bed sediment. The challenges of setting EQSs for sediment are mainly due to the variations in sediment physical and chemical characteristics, the lack of toxicity data, and identifying appropriate sediment sampling compartment (bed, bank and suspended sediment). This study aims to establish a sampling and analytical programme on a small river known to be contaminated with heavy metals, and investigate if there are any differences between measured concentrations of metals among the different sediment compartments. The processes affecting the distribution of selected heavy metals (Cd, Cu, Ni, Pb and Zn) in sediment was investigated by repeated sampling over a 20metre stretch of the Ravensbourne River, a tributary of



the River Thames located in South London. The results show that there were significant differences ( $p < 0.05$ ) in heavy metals concentrations between the different compartments. Most importantly, there were also significant differences ( $p < 0.05$ ) between different suspended tube samplers collected at the same time and location ( $\sim 0.75$  m apart on the same side, and  $\sim 7.5$  m apart on opposite sides of the river) in more than 60% of the sampling period. The total concentrations of metals were magnitudes higher in sediment compared to overlying water, and generally in the order  $\text{Zn} > \text{Pb} > \text{Cu} > \text{Ni} > \text{Cd}$ . Sequential extraction experiment to determine the bioavailable fraction was also carried out, and the results were compared with the organic matter content by loss on ignition and the x-ray diffraction experiment results. The significance of these results for setting EQSs and developing a monitoring programme are discussed.

**MP134 The importance of aqueous chemistry and water management on evaporative condenser performance** E.R. McKenzie, University of California Davis / Civil and Environmental Engineering. In hot and arid climates, evaporative condensers provide substantial energy savings compared to conventional forced air air conditioner systems. The evaporative condenser systems are being embraced by the utilities, however their longterm performance can be hampered by the formation of mineral scale (e.g. calcite). Calcium and magnesium carbonate are inversely-soluble minerals, meaning that their solubility decreases with increasing temperature; this is problematic for heat exchangers. Mineral scale impedes the flow of air, reduces heat transfer, and negatively impacts the performance of mechanical systems (e.g., pumps). Within the evaporative condenser, evaporation of the water results in an increase in the pH and an increased concentration of salts. The pH increase, in turn, increases the total carbonate concentration and shifts the carbonate distribution toward bicarbonate and carbonate. Concurrently, salts concentrations, including Ca and Mg, are increased; once the solubility limit is exceeded, only kinetic limitations impede precipitation reactions. Analysis of dissolved Ca and Mg indicate that Ca precipitation reactions (e.g.,  $\text{CaCO}_3$ ) occur more quickly than do Mg precipitation reactions. In water heaters, scale is observed to be  $\text{CaCO}_3$ , while mineral scale in evaporative coolers contains both Mg and Ca, and the mineral phases vary. Evaporative condenser water management strategies were employed to reduce mineral scale, and increase in the bleed (decreasing the salts residence time) improved unit performance. Physical water treatment devices, which claim to reduce the formation of mineral scale, are also being tested at this time as part of ongoing research. The physical water treatment devices that are being investigated include permanent magnets, electromagnets, and a vortex treatment devices.

**MP135 The science behind biodegradable claims for down the drain products** D. Versteeg, The Procter & Gamble Company / Environmental Science Department; S. Gimeno, The Procter & Gamble Company; T. Federle, The Procter & Gamble Company / Environmental Science Dept.. The biodegradability of formulations is increasingly being perceived as key in modern "sustainable" products. Despite the fact that biodegradability is becoming a common environmental claim on the packages of consumer goods products, there is no internationally agreed approach to assess the biodegradability potential of formulations (i.e. complex mixtures, preparations, finished product, whole-product formulations). This is likely to lead to misleading or even incorrect claims. Biodegradability assessments are typically conducted as input into the environmental risk assessments of individual organic substances. The existing standard procedures (e.g., OECD 301 series of Testing Guidelines) to assess ready biodegradability are appropriate to screen organic chemical substances. These procedures are not appropriate for heterogeneous mixtures such as formulations and the term biodegradation has no meaning for inorganic compounds (salts, water, etc.). Use of ingredient specific OECD 301 test data to establish overall biodegradability of a formulation is an open question and there is no agreed upon approach or set of rules. Various levels of biodegradability can be calculated depending on how one handles counterions in organic salts, hydrates, etc. and whether the calculation is based on organic carbon, weight percent, or mole percent. The pros and cons of each approach have been evaluated. Going forward, there is a need for an agreed, internationally accepted calculation method to report the biodegradability of whole product formulations in order to safeguard the credibility of environmental claims among the general public and consumers organizations.

**MP136 Trace Analysis of Compounds in Potable Water by On-Line SPE-LC/MS/MS** J. Stevens, Agilent Technologies, Inc. / Chemistries and Supplies Division; M. Woodman, P. Stephens, Agilent Technologies, Inc. / Chemical Analysis Group. Monitoring changes and trends of compounds in environmental samples at relevant concentrations it is essential to have techniques facilitating the detection of compounds at very low concentrations often down to ppt levels. To reach these low levels a high degree of concentration in the extraction step is required, commonly performed by LLE using solvents immiscible with water, such as dichloromethane, or by using off-line SPE columns. Large volumes are needed to reach these levels, the steps can be time-consuming, with many steps prior to instrument analysis, and subsequently have larger risk of analytical error. An alternative to these manual systems is automated systems using on-line SPE columns, also described as "trace enrichment cartridge" (TEC) when large volumes of water are passed through the cartridge; are gaining more and more interest. Automated on-line solid phase extraction (SPE) coupled with LC-MS/MS is enabling low ppt level detection of target analytes in potable and environmental waters. The availability of improved polymeric sorbents including mixed bed and copolymers which retain both moderately hydrophilic and hydrophobic analytes provides a cost effective solution to the quantification of pesticides, personal care pharmaceutical products (PCPP) and emerging contaminants. This application will explain how the on-line SPE process works, extraction capabilities, and the advantages to using on-line SPE for the extraction of very low concentrations of compounds from water sources.

**MP137 Urban impacts on regional background carbonaceous aerosols: investigation of seasonal urban outflow impacts** T. Barrett, Baylor University / The Institute of Ecological, Earth, and Environmental Sciences; A. Andersson, Stockholm University; R. Sheesley, Baylor University / Department of Environmental Science. Urban areas represent source regions of high interest in terms of their impact on surrounding populations and regional transport of pollutants. Aerosols are known to adversely affect the health of at-risk populations, such as the young and elderly, and chronic exposure to ambient concentrations of fine particulate matter ( $\text{PM}_{2.5}$ ) has been linked to cardiopulmonary diseases and lung cancer. However, it is difficult to ascertain the regional impact of urban outflow using only bulk chemical or meteorological data. A combination of techniques enables a more accurate assessment of the frequency, magnitude and character of the urban outflow at a background site. Our study site lies in central Texas, and is regularly 12-24h downwind of metropolitan areas including the Dallas-Fort Worth metropolitan area (4.2 million) and the Houston metropolitan area (4 million). Back trajectory analysis (BT), chemical characterization (organic and elemental carbon – OCEC, water soluble organic carbon – WSOC, and inorganic ions including  $\text{SO}_4$  and  $\text{NO}_3$ ), mass absorption cross-section (MAC) and the light attenuation coefficient (ATN) will be presented for a yearlong sampling campaign (May 2011- June 2012). Correlations among these factors (BTs, OCEC, WSOC,  $\text{SO}_4$ ,  $\text{NO}_3$ , MAC and ATN) will be used to characterize urban PM and potential emission sources from these two metropolitan centers. Seasonal differences in the OCEC, WSOC,  $\text{SO}_4$ ,  $\text{NO}_3$ , MAC and ATN transported from these urban centers will be explored in greater detail during a summer and winter field intensives. Preliminary data (May 2011-Feb 2012) indicates EC to OC ratio of 0.066 with an  $r^2 = 0.46$ . Seasonal trends show a higher EC/OC ratio for the winter (0.06), which is dominated by N and NW winds, than for the summer (0.04), dominated by S and SE winds.

**MP138 Using  $k_0$ -NAA to Assess Sediment Quality in Causeway Lake (Queensland, Australia)** V. Vicente-Beckett, Central Queensland University / Centre for Environmental Management; J. Bennett, Australian Nuclear Science and Technology Organisation / Neutron Activation Group – Nuclear Operations. Causeway Lake is a popular recreational area for water sports. It was created when a bridge, a weir and a causeway were built across the Mulambin estuary in 1939. The current water depth varies from 0.5-1.3 m, versus the original 2-3 m depth. The Mulambin area has recently seen residential and other developments. The sedimentation rates in the lake were estimated recently at  $1000 \text{ m}^3/\text{y}$  transported into the lake by tidal currents and  $1500 \text{ m}^3/\text{y}$  from the catchment. This study aimed to determine metal concentrations in Causeway Lake sediments using instrumental neutron activation analysis (INAA) and the  $k_0$ -method of standardisation, and to assess the sediment quality using the ANZECC sediment quality guidelines. NAA is attractive because of its minimal sample preparation requirements and multi-element detection capability; furthermore a single monitor is sufficient

for the *k0*-method to determine the concentration of all detectable elements in a sample. To check the reliability of the *k0*-NAA method, BCSS-1 (estuarine) and PACS-2 (marine) certified sediment reference materials were analysed. The *k0*-NAA analyses of the Causeway Lake sediments were also compared with ICP-OES or ICP-MS analyses. The study used the new 20 MW OPAL research reactor operated by ANSTO. At the INAA irradiation positions used the neutron flux was around  $1.5 \times 10^{13} \text{ cm}^{-2} \text{ s}^{-1}$  and was well thermalised (thermal/epithermal flux ratio >1000). Irradiations were carried out in both the short and long residence time facilities to maximise the number of quantifiable elements. The neutron flux monitor for *k0*-NAA was the reference material IRMM-530R which contained 0.1 % gold. Gamma-ray spectra were analysed using HyperLab and elemental concentrations calculated using Kayzero for Windows. The ratios of the *k0*-NAA data to the reference sediments' certified values were generally 0.9-1.1 indicating reliability of the method. At least 30 elements (excluding Ag, Cd, Cu, Ni and Pb) were detected in the Causeway Lake sediments (both surficial sediments and core slices) but only eight elements were compared because of the limited ICP-OES/ICP-MS dataset. The table (ratio of *k0*-NAA to ICP-OES/ICP-MS data) shows comparable average concentrations, except for Cr and Sb. No significant metal contamination was indicated, although As and Cr appeared enhanced in some sediments. Al As Ca Cr Fe Mn Sb Zn 1.13 0.96 1.07 1.40 1.03 1.08 1.23 1.06

**MP139 Water quality of in-land surface waters in the UAE** S.L. Knuteson, American University of Sharjah / Biology, Chemistry and Environmental Sciences; A. Veroni, American University of Sharjah. Although the United Arab Emirates is considered a desert region, in the mountainous areas, there are many sources of fresh water, mostly associated with groundwater sources. Many of these areas involve waterfalls, streams, pools of various sizes and/or falaj (irrigation) systems for distributing water to the local residents developed as much as a thousand years ago. As such, they have been extremely utilized in many different ways such as domestic waters, agricultural irrigation sources and touristic uses such as swimming. Some areas are within villages or palm plantations; while some of them are remote and only accessible by four wheel drive vehicles and used for grazing or tourism. A simple drive through these areas can show significant environmental impacts from tourism, local residents, and grazing cattle such as goats, donkeys and camels. This project was designed to characterize the water quality of seven wadi systems in the Hajar Mountains of the UAE and consider the risk to human and ecological health based on standard water quality objectives from local and the World Health Organization (WHO). Data suggests the water was typical of fresh water ecosystems as other studies done in the United Arab Emirates suggested. One sampling area, however, showed extreme signs of eutrophication, possibly due to location within a wadi running through Hatta city limits with impacts from local residential areas on septic systems and wandering animals. Water from the Hatta city wadi had chemical and biological parameters that did not meet the drinking, swimming or agricultural water quality regulations of the UAE or WHO. Elsewhere throughout the sampling areas, the main issues found with the water were the high levels of coliforms and *E. coli* found in the waters, with levels above 1000 coliforms/100mL in many samples and one as high as 22000 coliforms/100mL in a wadi with a rookery. Therefore, most of the waters were not safe for agricultural use on food crops, swimming or drinking when compared to water quality regulations due to bacterial contamination.

**MP140 When Is Enough Sampling Enough? — An ESHIA Survey** M.F. Shibata, N.T. Hausmann, ERM, Inc.. An Environmental, Social, and Health Impact Assessment (ESHIA) was being prepared for a development project in Africa to identify, analyze, and develop measures that would enhance benefits, and avoid or mitigate environmental, social, and health impacts. In support of this ESHIA, a baseline survey was planned for three populations of interest: (i) physically displaced, (ii) economically impacted, and (iii) otherwise impacted populations. Households within each population of interest would be queried for demographical, educational, economic, and health-related information. Population attributes collected as part of this baseline survey would consist of continuous, scaled continuous, and categorical data types. A key question was how many households needed to be surveyed to obtain data that would be representative and could be used to make sound inferences for each population. Factors used to establish a statistically defensible sample size included: (i) level of significance [or Type I error], (ii) false negative error [or Type II error], (iii) variability within

a population, and (iv) size of the observed effect or acceptable margin of error. To assist designers in scoping the survey effort, sample sizes sufficient to characterize population attributes were plotted for different levels of significance and different margins of error. Changes in the relative margin of error were also plotted as a function of sample size. These plots allowed designers to see how confidence in characterizing population attributes, risks in exceeding margins of error, or ability to discern differences changed with sample size. Points of diminishing return were a key consideration when establishing the sample size for the survey. Categorical data types generally drove minimal sample sizes. When characterizing attributes, changes in acceptable margin of error had a greater effect on samples size as compared to changes in level of significance.

**MP141 Using rapid toxicity assessments to guide the characterization of complex matrix interactions: Analysis of groundwater in the Barnett shale** Z. Hildenbrand, B. Fontenot, L. Hunt, D.D. Carlton, H. Oka, University of Texas at Arlington; D. Hopkins, Geotech Environmental, Inc; B. Bjorndal, A. Osorio, Assure Controls; K. Schug, University of Texas at Arlington. Governmental policy regulates surface and public drinking water quality; but no authority monitors groundwater cleanliness, which is approximately 15% of U.S. supply. The prevalence of hydraulic fracturing and the expansion of natural gas extraction warranted an assessment of possible environmental impacts, particularly as they pertain to groundwater contamination. In this study we assessed the water quality of 100 private water wells in and around the Barnett Shale of Texas. Due to the recent commercial availability of a packaged live organism in kit form which can provide a rapid initial reading of any contaminant at a biologically harmful level (at a low cost) the researchers utilized the ability to have a comprehensive first measurement, then determine if water quality was normal or potentially compromised. Historically, live species testing has been too complex or expensive to consider using biological indicators as the first "sensor" in the analysis process. Using this technique (ASTME1924, with tradename QwikLite™) biological toxicity was detected in 24% of our samples. We found that the matrix of each water sample could either attenuate or accentuate the toxicology response of the respective contaminants and that concentrations of each toxicant correlated with toxicity upon fractionation. Subsequent chemical analyses revealed that heavy metals, often associated with natural gas drilling flow back water, and total dissolved solids (TDS), reach the highest levels in domestic wells located less than 3 km from natural gas drilling sites, with no discernible geospatial pattern of toxicity. Methanol and ethanol were also detected in 29% of samples, providing additional indication of industrial activity. The tools and techniques used in the investigation proved to be reliable measurement techniques for assessment of exogenous substances associated with the hydraulic fracturing activities. Collectively we found evidence of biological toxicity, elevated levels of heavy metals, elevated total dissolved solids (TDS) levels, methanol, ethanol, and potentially exogenous, unidentified chemical compounds in several domestic water wells. These data provide an initial characterization of groundwater quality in the Barnett Shale, in addition to delineating the relationship between aquatic matrix interactions and toxicant bioavailability.

**MP142 Accumulation feature of trace elements in mass-stranded harbor seals (*Phoca vitulina*) in the North Sea coast in 2002** T. Agusa, Center for Marine Environmental Studies (CMES) / Shimane University, Ehime University / Center for Marine Environmental Studies (CMES); S. Yasugi, A. Iida, T. Ikemoto, Ehime University / Center for Marine Environmental Studies (CMES); Y. Anan, Showa Pharmaceutical University; T. Kuiken, A.D. Osterhaus, Erasmus Medical Center; S. Tanabe, H. Iwata, Ehime University / Center for Marine Environmental Studies (CMES). Body distribution and growth- and nutritional status-dependent accumulation of 21 trace elements were investigated in harbor seals (*Phoca vitulina*) stranded in the North Sea coast in 2002. Higher concentrations and burdens of Mn, Se, Mo, Ag, Sn, Hg, and Bi in the liver, Cd in the kidney, As in the blubber, and Co, Sr, and Ba in the bone were observed. Significant positive correlations of hepatic concentrations of Se, Mo, Ag, Cd, Sn, Hg, Tl, and Bi with standard body length were found, while significant negative relationships were detected for Mn, As, Rb, Sr, and Sb. Concentrations of Co, Se, Sr, Sn, Hg, and Bi in the liver, V, Sr, Ag, Sn, and Hg in the kidney, V, Mn, Co, Rb, Sr, Sn, Ba, and Pb in the blubber increased with a decrease of the blubber thickness of stranded harbor seals, indicating enrichment of these elements in the target tissue by emaciation. To clarify whether trace element accumulation in harbor seals is linked with phocine distemper virus (PDV)

infection, concentrations of trace elements in the liver of PDV infected seals in 2002 were compared with those of non-infected seals collected in 1999. By the analysis of logistic regression analysis, the increased Rb and decreased Cu levels in the liver of harbor seals were significantly associated with PDV infection.

**MP143 Climate change impacts on contaminants in a beluga whale population that segregates into sea ice defined habitats for foraging** L.L. Loseto, Fisheries and Oceans Canada / Department of Zoology, Fisheries and Oceans Canada / Freshwater Institute; M. Noel, Institute of Ocean Sciences, Fisheries and Oceans Canada, University of Victoria / School of Earth and Ocean Sciences, University of Victoria; P.S. Ross, Fisheries and Oceans Canada / Institute of Ocean Sciences. Recent years have seen record lowest sea ice extent in the arctic, with historic lows in 2007 and 2011. While the implications for biota are unclear, they may be expected to be dramatic for those species that rely on sea-ice associated food webs. For the Beaufort Sea beluga whales that rely on sea ice for prey such as Arctic cod, these recent climate driven-changes may alter the quantity and quality of available prey. Such altered food webs have likely modified the exposure of beluga whales to a variety of persistent organic pollutants (POPs) such as PCBs and PBDEs. In addition, changes in sea ice extent and structure is expected to alter the transport and fate of POPs in the arctic environment, the implications of which are far from clear for beluga whale food webs. With the renewed interest from the oil and gas industry in developing projects within the summer feeding habitat of beluga whales, knowledge on current conditions and delineating climate change driven impacts is needed for better decision making. We hypothesize that food web changes related to a receding sea ice edge may impact beluga health by decreasing food quantity and quality defined by energetic content and by contaminants. Using dietary biomarkers such as fatty acids and stable isotopes, we characterized beluga diet among size defined foraging groups across years to test for temporal variations. These trends were compared with contaminant levels of PCBs and PBDEs among the foraging groups to assess changes in dietary exposure in relation to diet quality. Results revealed a lack of relation in PCBs and PBDEs with age, rather PCB and PBDE concentrations fit a dietary response to size-defined beluga groups. However, dietary profiles among these groups changed over time, suggesting that an altered food web structure for beluga whales did not noticeably alter their exposure to contaminants. We explore the implications of a changing diet on both nutritional and contaminant exposure consequences using likely prey items. Trends in PCB and PBDE concentrations prey and beluga size-defined feeding groups highlight the need for PCB metabolism and beluga metabolic processes to be investigated. Continued monitoring of long-lived beluga whales in the Beaufort Sea will help provide insight into the consequences of climate change on their diet, and will support the provision of advice on means and methods of localized oil and gas development activities.

**MP144 Reconstruction of Pesticide, PCB, and PBDE Profiles in Gray and Blue Whale Earplugs** E. Robinson, Baylor University / Environmental Science, Baylor University / The Institute of Earth, Ecological, and Environmental Sciences, Baylor University / The Institute of Ecological, Earth, and Environmental Sciences; S. Trumble, Baylor University / Department of Biology; M. Berman, Santa Barbara Museum of Natural History; C. Potter, Smithsonian Institution; S. Usenko, Baylor University / Department of Environmental Science. Contaminant trends and profiles have been reconstructed from matrices, such as sediment and ice cores, have provided a wealth of information regarding contaminant behavior and environmental fate. Like all mammals, whales excrete wax into their ear canals; however, select whale species accumulate their earwax over their lifetime (~20 to 100 years) forming an earwax plug. Over the past 60 years, many earwax plugs have been archived in some of the world's most prestigious natural history museums. Earwax plug layers have been used to estimate the organism's age similar to aging tree rings. Whale earplugs may represent a unique mammalian matrix that enables the analysis of recent and historic contaminant concentrations within the individual earplug layers. Whale earplugs were investigated to determine if they are viable matrices for recording contaminants in marine ecosystems. Using enhanced pressurized liquid extraction and gas chromatography-mass spectrometry electron capture negative ionization and electron impact modes, we analyzed for pesticides, PCBs, and PBDEs in a historically archived gray whale (*Eschrichtius robustus*) earplug (harvested in 1969) and a recently harvested blue whale (*Balaenoptera musculus*) earplug (harvested in 2007). Blue whale blubber was also analyzed

for pesticides, PCBs, and PBDEs for comparison to the earplug concentrations and contaminant profiles. The blue whale earplug and blubber had similar contaminant profiles; although, contaminant concentrations were an order of magnitude higher in the blubber than the earplug. The gray and blue whales were harvested along the Californian coast. Comparisons of historic versus recent contaminant concentrations and profiles were performed. Trans- and cis-chlordane were identified in the gray whale but were not identified in blue whale earplugs. Trans- and cis-nonachlor was detected in both the gray and blue whale earplugs. PCBs were detected in both whale earplugs, while PBDEs were only detected in the blue whale earplug.

**MP145 Vitamins as biomarkers: distinguishing between toxicity and feeding ecology in western Arctic beluga whales** J.W. Desforges, University of Victoria / Earth and Ocean Science; P.S. Ross, Fisheries and Oceans Canada / Institute of Ocean Sciences; L.L. Loseto, Fisheries and Oceans Canada / Department of Zoology, Fisheries and Oceans Canada / Freshwater Institute. Beluga whales are long lived, charismatic, high trophic level predators and are an important food source and cultural icon for Inuvialuit people. Because of their important role in Arctic ecosystems they can be used as sentinel species, providing valuable insight into general ecosystem health. Beluga are increasingly exposed to biological and environmental stressors, and of particular concern is exposure to persistent, bioaccumulative and toxic (PBT) chemicals. Biomarkers are used as sensitive indicators of toxic exposure to chemicals and potential health impacts in wildlife. Vitamin A and E are essential nutrients and have been shown to be viable biomarkers of exposure in birds and marine mammals. In seals and cetaceans, increased concentrations of PBT chemicals is typically linked with lower tissue vitamin A and increased vitamin E levels; however, several studies have uncovered the opposite trend. The discrepancy between studies is likely due to confounding biological processes controlling the uptake and elimination of these dietary vitamins. As such, we've established a temporal food web study in Arctic Canada to explore vitamin and contaminant dynamics. In collaboration with Inuvialuit hunters, we collected samples from 67 healthy beluga over four years (2007-2010) as well as five different prey species from the Mackenzie Delta and Beaufort Sea. We quantified 138 PCB and 30 PBDE congeners and over 18 vitamin A and three vitamin E compounds in various beluga tissues and prey. Our results show that sex, reproductive status, and feeding ecology shape contaminant patterns, but also influence vitamin levels in beluga. Preliminary results suggest that both contaminants and feeding preferences are affecting vitamin concentrations in these free-ranging marine mammals, but additional research is needed to shed mechanistic light on these observations. Results highlight the importance of considering confounding factors when using vitamins as biomarkers of PBT contamination in beluga whales.

**MP146 What ringed seals are telling us about temporal trends of persistent organic pollutants in the Canadian Arctic** D. Muir, Environment Canada / Aquatic Ecosystem Protection Research Division, Environment Canada / Aquatic Contaminants Research Division; X. Wang, A. Sett, Environment Canada / Aquatic Contaminants Research Division; E. Barresi, E. Sverko, Environment Canada / National Laboratory for Environmental Testing; S. Ferguson, Dept of Fisheries and Oceans / Arctic Aquatic Research Division; A. Fisk, University of Windsor / GLIER; M. Kwan, Nunavik Research Centre. The ringed seal is the most abundant Arctic pinniped with a circumpolar distribution and has been a key biomonitoring animal for examining spatial and temporal trends of persistent organic pollutants (POPs) since the 1970s. The goal of this ongoing study is to determine temporal trends of legacy POPs and new/emerging POPs in Canadian Arctic ringed seals using annual collections in Hudson Bay (Arviat), in Lancaster Sound (Resolute), in southeastern Beaufort Sea (Sachs Harbour) and Cumberland Sound (Pangnirtung). The study builds on results for legacy POPs going back to the 1980s. Results for new POPs, polybrominated diphenyl ethers (PBDEs), hexabromocyclododecane (HBCD), perfluorinated chemicals (PFCs), and endosulfan, along with carbon and nitrogen stable isotope data, have been added to samples collected since 2001 and on selected archived samples from the 1970s and 1990s. Sample collections consisting of 10 to 25 adult ringed seals (blubber, liver, muscle, tooth/lower jaw for aging) are carried out by hunters each year (June-October) as part of their traditional hunting. Sample analysis follows previously published methods for neutral POPs in blubber and liver (for PFCs only). Only blubber of females and juveniles are analysed to limit the influence of age. Results show that there are declining trends for legacy POPs in all locations with the relative magnitude



of  $\Sigma\text{DDT} > \alpha\text{HCH} > \Sigma\text{10PCB} > \Sigma\text{CHL}$ . Largest declines are in Hudson Bay ( $\Sigma\text{10PCB} = 5\%/yr$ ;  $\Sigma\text{DDT} = 7\%/yr$ ) possibly reflecting proximity to source regions in eastern and central North America. PBDEs increased in concentrations in the 1990s to 2010 at Resolute and Sachs Harbour while in Hudson Bay they have declined over the period 2005-2011. PFOS and perfluorocarboxylates also increased until 2003-2005 in all locations but have declined over 2005-2011. Endosulfan, hexabromocyclododecane and bis(tribromophenoxy)ethane are present at low concentrations in seal blubber (0.01-2.0 ng/g) and appear to be increasing in concentration over the period 2005-2011. The trends for new POPs differ from those in Greenland particularly for PFCs. Proximity to source regions and the influence of ocean transport through the Canadian archipelago may be the reasons for these differences. Continued annual sampling is improving the statistical power of the study and enabling testing of factors influencing trends of POPs and Hg including climate, diet and changes in global emissions.

**MP147 Assessing the Environmental Fate and Effects of Insensitive Munitions in Terrestrial Environments** J.G. Coleman, US Army Engineer Research and Development Center, US Research and Development Center / Environmental Laboratory, US Army Engineer Research and Development Center / Research Biologist, US Research and Development Center / Research Biologist; J. Seiter, U.S. Army Corps of Engineers / Research Physical Scientist; A.J. Bednar, US Army Engineer Research and Development Center / Environmental Laboratory; A.L. Russell, Badger Technical Services. The U.S. Army has sought to replace 2,4,6-trinitrotoluene (TNT) with a new generation of insensitive munitions (IM) aimed at reducing sensitivity to external stimuli without loss of energetic performance; although IMs have received sanction for military use, little information is available on the implications of these emerging materials in terrestrial environments. IMX-101 has been approved as the main fill ingredient in M795 155-millimeter artillery munitions. This relatively new IM mixture consists of 40% 2,4-dinitroanisole (DNAN), 20% 3-nitro-1,2,4-triazol-5-one (NTO), and 40% nitroguanidine (NQ). Our objective is to address the interesting challenge of mixture characterization, environmental impacts, and toxicity of IMX-101 and its components in the terrestrial environment. Initially, IMX-101 was tested for stability in multiple aqueous solutions at a 5 ppm concentration with varied pH and exposure to light, and monitored over 28 days for degradation potential ( $n=3$ ). Post 28 days, high performance liquid chromatography analysis (HPLC) indicated that IMX-101 remained stable in all solutions. Bench-scale soil batch studies were employed to determine environmentally relevant rates of IMX-101 retention in terrestrial systems. We assessed retention rates of IMX-101 in solution (10-50 ppm) in a field collected and artificial soil over 24 hours ( $n=3$ ). Following HPLC analysis of solutions in soil reactions, results indicated up to 50% of DNAN retention, while NQ retention was 25 % or less in soils. This data may indicate that DNAN sorbs more readily to soils; and therefore is more bio-available to terrestrial biota than other compounds present in IMX-101 mixtures. Soil mobility results are being combined with bioaccumulation and toxicity screening assays utilizing the earthworm, *Eisenia fetida* to predict rates of uptake and potential impact of IMX-101 in terrestrial invertebrates. Ultimately, the proposed efforts will provide both Army researchers and range managers with previously unavailable information on bioaccumulation pathways, degradation, and mobility of DNAN and IMX-101.

**MP148 Aqueous and Soil Toxicity and Bioaccumulation of 2,4-dinitroanisole DNAN in the Earthworm *Eisenia fetida*** G. Lotufo, US Army Engineer Research and Development Center / Environmental Laboratory; J. Coleman, A. Harmon, S. Brasfield, U.S. Army ERDC. The environmental impacts of munitions and explosives of concern are difficult to predict due to the limited information available and the potential for rapid degradation to breakdown products. The insensitive explosive, 2,4-dinitroanisole (DNAN), is fully qualified and in use as a replacement for trinitrotoluene (TNT). DNAN was less toxic than TNT to aquatic receptors and a potential breakdown product in alkaline environments, 2,5-methoxy nitrophenol, has resulted in greater toxicity relative to the parent material to earthworms and aquatic invertebrates. Because of the scarcity of data on DNAN toxicity and bioaccumulation in earthworm, we conducted aqueous phase toxicity and toxicokinetic exposures using *Eisenia fetida*. Toxicokinetic studies were conducted in aqueous phase to determine basic kinetic parameters (ratios of uptake, depuration, biotransformation, and bioconcentration). Bioaccumulation factors (BAFs, ratio of tissue to soil concentrations) were derived using adult earthworms exposed to sublethal concentrations of DNAN

in natural soils with contrasting physicochemical properties. Correlations between soil and porewater concentrations are used to investigate the role of various factors (e.g., clay content, organic carbon, pH) towards controlling DNAN bioavailability. The ability of soil porewater to predict earthworm bioaccumulation of DNAN and its transformation products will be assessed in future work. Lethal toxicity data generated after 4 and 10 days of aqueous exposure and the kinetics parameters uptake and depuration rates, bioconcentration factor and biotransformation potential will be presented.

**MP149 Determining Adsorption-Desorption Kinetics of Munitions Constituents with Nitrocellulose** M. Simini, U.S. Army Edgewood CB Center, U.S. Army Edgewood Chemical Biological Center / Environmental Toxicology, U.S. Army Edgewood CB Center / RDCB-DRT-E / E5641; R.T. Checkai, Edgewood Chemical Biological Center; R.G. Kuperman, U.S. Army Edgewood Chemical Biological Center / Environmental Toxicology; R. Gonzalez, University of Delaware / Environmental and Civil Engineering; H.E. Allen, University of Delaware / Dept. Civil & Environmental Engineering; D. DiToro, University of Delaware / Civil and Environmental Engineering Dept.. Development of effective technologies for predicting the fate and transport of munitions constituents (MC) at explosives-contaminated sites is needed to sustain operational training and testing ranges. Dissolution of MC from propellant particles is a concern at these sites. The presence of the nitrocellulose (NC) matrix complicates modeling of MC release from propellant, and into soil solution. In addition to the dissolution of the soluble components, the role of adsorption to, and desorption from, the NC and the swelling due to the presence of water need to be taken into account. No adsorption/desorption kinetic model of the MC to NC presently exists. The present studies investigated the adsorption-desorption kinetics of nitroglycerin (NG) and 2,4-dinitrotoluene (2,4-DNT) with nitrocellulose (NC, 12.3% N). NC was wetted to saturation for five days, then a solution amended with known concentrations of NG and 2,4-DNT was added. Replicated samples were extracted from the solution and from the NC at timed intervals, ranging from 15 minutes to 72 hours and analyzed by HPLC. Rate of adsorption and four consecutive desorptions were determined. For adsorption from a non-replenished source, most of the adsorption occurred within the first few hours. Desorption rates for the four consecutive desorptions for each MC were compared during the 72-hour duration. Adsorption and desorption isotherms were then determined for NG and 2,4-DNT. Results from these studies, plus results from replenished source studies, which are underway, will be compared and discussed in relation to kinetic models developed in collaborative studies. These studies were supported by project number ER-1688 of the Strategic Environmental Research and Development Program (SERDP).

**MP150 Dual mode mitigation of transport of 2,4,6-trinitrotoluene and heavy metals by monopotassium phosphate in military firing range** S. Im, Seoul National University / Dept. of Civil and Environmental Engineering; J. Jung, Seoul National University / Department of Civil and Environmental Engineering; G. Lee, Seoul National University / Dept. of Civil and Environmental Engineering; K. Nam, Seoul National University / Department of Civil and Environmental Engineering, Seoul National University / Dept. of Civil and Environmental Engineering. In active military firing ranges, it is very important to mitigate the transport of 2,4,6-trinitrotoluene (TNT) and heavy metals such as lead (Pb) and copper (Cu) into the surface water, usually located close to the firing ranges. We hypothesized that application of monopotassium phosphate ( $\text{KH}_2\text{PO}_4$ ) may attain the reduction of transport of both contaminants simultaneously, which was tested with a field soil collected from a firing range. The soil texture was sandy loam. Batch sorption test results showed that, in the absence of monopotassium phosphate, TNT sorption was negligible and about 60% of initial Cu concentration (767mg/kg-soil) was found in aqueous phase. When 0.1 M monopotassium phosphate was added, however, more than 40% of initial TNT (30mg/kg-soil) was sorbed to soil and only 28% of initial Cu was found in aqueous phase. Such dual mode action was more evident at a higher potassium monophosphate concentration (0.5 M). Freundlich coefficient of TNT in the presence of 0.1 M  $\text{KH}_2\text{PO}_4$  was 5.74 L/kg, and increased to 7.91 L/kg when 0.5 M of  $\text{KH}_2\text{PO}_4$  was present. Cu concentration in aqueous phase was also reduced to 6% of initial concentration in the presence of 0.5 M of  $\text{KH}_2\text{PO}_4$ . It is likely that sorption of TNT was enhanced by  $\pi$ - $\pi$  interaction between clay surfaces and the compound and Cu reduction in aqueous phase resulted from the formation of metal phosphate precipitates. The findings suggest that the transport of TNT and

Cu into the environment can be mitigated by KH<sub>2</sub>PO<sub>4</sub> application. Further study is underway to investigate the nature of dual mode interactions and to determine the means and concentration of appropriate monopotassium phosphate application.

**MP151 Aqueous and Soil Toxicity and Bioaccumulation of 2,4-dinitroanisole (DNAN) in the Earthworm *Eisenia fetida*** G. Lotufo, US Army Engineer Research and Development Center / Environmental Laboratory; J. Coleman, U. Army ERDC; A. Harmon, S. Brasfield, U. S. Army ERDC. The environmental impacts of munitions and explosives of concern are difficult to predict due to the limited information available and the potential for rapid degradation to breakdown products. The insensitive explosive, 2,4-dinitroanisole (DNAN), is fully qualified and in use as a replacement for trinitrotoluene (TNT). DNAN was less toxic than TNT to aquatic receptors and a potential breakdown product in alkaline environments, 2,5-methoxy nitrophenol, has resulted in greater toxicity relative to the parent material to earthworms and aquatic invertebrates. Because of the scarcity of data on DNAN toxicity and bioaccumulation in earthworm, we conducted aqueous phase toxicity and toxicokinetic exposures using *Eisenia fetida*. Toxicokinetic studies were conducted in aqueous phase to determine basic kinetic parameters (ratios of uptake, depuration, biotransformation, and bioconcentration). Bioaccumulation factors (BAFs, ratio of tissue to soil concentrations) were derived using adult earthworms exposed to sublethal concentrations of DNAN in natural soils with contrasting physicochemical properties. Correlations between soil and porewater concentrations are used to investigate the role of various factors (e.g., clay content, organic carbon, pH) towards controlling DNAN bioavailability. The ability of soil porewater to predict earthworm bioaccumulation of DNAN and its transformation products will be assessed in future work. Lethal toxicity data generated after 4 and 10 days of aqueous exposure and the kinetics parameters uptake and depuration rates, bioconcentration factor and biotransformation potential will be presented.

**MP152 Lipophilicity of Munitions Compounds: Octanol:water (K<sub>ow</sub>) vs. Solid Supported Liposome:water (K<sub>lipw</sub>) Partitioning** A.B. Goins, Badger Technical Services; D.R. Johnson, U.S. Army Engineer Research and Development Center / Environmental Laboratory, U.S. Army / CEERD-EP-R, US Army Engineer Research and Development Center / Environmental Laboratory, US Army Engineer Research & Development Center / Environmental Laboratory; C.Y. Ang, Badger Technical Services; A.J. Bednar, US Army Engineer Research and Development Center / Environmental Laboratory; T.M. Reese, Jackson State University; F.C. Hill, US Army Engineer Research and Development Center / Environmental Laboratory. Bioaccumulation of organic molecules into fat can be predicted by chemical lipophilicity which is measured by the octanol:water partition coefficient (K<sub>ow</sub>). However, lipophilicity using octanol micelle as a lipid membrane surrogate is not an accurate reflection of cellular bilayer structure. Newly developed methods use the liposome:water partition coefficient (K<sub>lipw</sub>) and phosphatidylcholine (PC) instead of octanol to more accurately represent the capacity of membrane lipids to accumulate neutral organic chemicals. In this study, a new technique for measuring K<sub>lipw</sub> using solid-supported PC liposomes attached to silica beads (TRANSIL® Sovicell, Leipzig, Germany) was used to assess the membrane affinity (i.e., K<sub>lipw</sub>) of 15 munitions compounds: nitroaromatic munitions (2,4,6-trinitrotoluene [TNT], 2,4-dinitrotoluene [2,4-DNT], 2,6-dinitrotoluene [2,6-DNT], 1,3,5-trinitrobenzene [TNB], 2,4-dinitroanisole [DNAN]), cyclic nitramine munitions (hexahydro-1,3,5-trinitro-1,3,5-triazine [RDX], octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine [HMX]), hexanitrohexaazaisowurtzitane [CL-20]), and new munitions (5-nitro-2,4-dihydro-3H-1,2,4-triazol-3-one) [NTO], (2,2-dinitro-1,1-ethenediamine) [FOX-7]. Measured log K<sub>lipw</sub> was compared to values derived from log K<sub>ow</sub> values obtained from both literature and computational sources (KOWWIN, ACD). Difference between mean log K<sub>ow</sub> and log K<sub>lipw</sub> (1.23 vs. 2.35) was significant (p1 log unit higher than predicted by K<sub>ow</sub> was seen for all cyclic nitramine munitions and the emerging explosive FOX-7. Results indicate a general trend for different measures of lipophilicity in descending order of apparent accuracy: K<sub>lipw</sub>(TRANSIL®) > K<sub>lipw</sub>(K<sub>ow</sub>) > K<sub>ow</sub> (literature) ≥ K<sub>ow</sub> (ACD) > K<sub>ow</sub> (KOWWIN). These data support the use of K<sub>lipw</sub> over K<sub>ow</sub> in predicting bioaccumulation in risk assessment models.

**MP153 Bioavailability implications of nanomaterials used in munition formulations** J.K. Stanley, U.S. Army Engineer Research and Development Center / Environmental Laboratory, U.S. Army Engineer Research And Development Center / Environmental Laboratory, U.S. Army Engineer Research and Development Center / CEERD-EP-R; A.J. Kennedy, US Army Engineer Research and Development Center, CEERD-EPR; J. Brame, Rice University; C.D. Lounds, Badger Technical Services; N.E. Harms, U.S. Army Engineer Research and Development Center; A.J. Bednar, US Army Engineer Research and Development Center / Environmental Laboratory. The large relative surface area and reactivity of nanomaterials may impact the transport of metal and organic toxic contaminants in the environment. Nanomaterials such as nano aluminum and carbon nanotubes (CNTs) are used in military energetic formulations as oxidizers and stabilizers, respectively. Such applications of nanomaterials are predicted to increase in the future. The purpose of the present research was to assess the effects that interaction with these nanomaterials have on the bioavailability of other munition constituents (MC). RDX (cyclotrimethylenetrinitramine) was chosen as a model energetic MC, and tungsten (W) was chosen as a model metal MC. As an initial indicator of the capacity of nanomaterials to affect MC fate, the ability of nano aluminum oxide (Al<sub>2</sub>O<sub>3</sub>) and CNTs to bind to and remove RDX and W from solution was determined. Significant reductions in aqueous concentrations of RDX were observed in the presence of CNTs and significant reductions in aqueous concentrations of W were observed in the presence of nano Al<sub>2</sub>O<sub>3</sub> in aqueous sorption studies performed utilizing 500 mg/L nanomaterial and 2 mg/L MC. Since other published research has demonstrated the ability of nanomaterials to either increase or decrease bioaccumulation of sorbed contaminants, ongoing research will be presented on the effects that these interactions have on *Corbicula fluminea* (Asiatic clam) and *Daphnia magna* bioaccumulation when nanomaterials and MCs are present in mixtures.

**MP154 Toxicity and bioaccumulation of the energetics TNT and RDX to Northern Leopard Frog tadpoles** J.K. Stanley, U.S. Army Engineer Research and Development Center / Environmental Laboratory, U.S. Army Engineer Research And Development Center / Environmental Laboratory, U.S. Army Engineer Research and Development Center / CEERD-EP-R; K.A. Gust, ERDC-EL-EP-P / Environmental Laboratory, US Army, Engineer Research and Development Center, US Army Engineer Research & Development Center, ERDC-EL-EP-P; G. Lotufo, US Army Engineer Research and Development Center / Environmental Laboratory; J.M. Biedenbach, U.S. Army Engineer Research and Development Center / Environmental Laboratory. Bullfrog (*Rana catesbeiana*) tadpoles have recently been demonstrated to exhibit high sensitivity to chronic exposure to energetic compounds such as trinitrotoluene (TNT). This is of particular concern due to widespread amphibian decline across the globe and because there are examples of threatened and endangered amphibians on military training ranges in the United States such as the gopher frog, *Rana capito*. With a future goal of better understanding the mechanisms of tadpole sensitivity to energetics, the purpose of the present study was to begin this process by determining the sensitivity of Northern Leopard Frog (*Rana pipiens*) tadpoles, a close phylogenetic relative of *R. capito*, to the current use energetics TNT and RDX (cyclotrimethylenetrinitramine). 96-h and 10-d toxicity (survival) tests were performed for TNT and RDX, respectively. In addition, swimming behavior in alternating periods of light and darkness was assessed at the end of RDX exposure using digital tracking behavioral software. Bioaccumulation (96-h) and elimination (96-h) kinetics studies were also performed with TNT and RDX. The observed 96-h LC50 for TNT exposure was 4.41 mg/L TNT and the 10-d RDX NOEC and LOEC were 12.3 mg/L and 25.3 mg/L, respectively. Startle response to light was shown to be impacted in fish exposed to 25.3 mg/L RDX. In the bioaccumulation study, apparent steady state tissue concentrations were obtained within hours for TNT and RDX with bioconcentration factors of 4.9 and 0.9 for TNT and RDX, respectively. Future studies will include long-term chronic (90-d) exposures of *R. pipiens* to TNT and RDX with intensive examination of gene expression changes in response to energetic exposure.

**MP155 Toxicity and low concentration growth increases of green algae in response to exposure to the new insensitive high nitrogen energetic TAGMNT** J.K. Stanley, U.S. Army Engineer Research and Development Center / Environmental Laboratory, U.S. Army Engineer Research And Development Center / Environmental Laboratory, U.S. Army Engineer



Research and Development Center / CEERD-EP-R; J.M. Biedenbach, U.S. Army Engineer Research and Development Center / Environmental Laboratory; A.L. Russell, U.S. Army Engineer Research and Development Center / Environmental Laboratory; A.J. Bednar, US Army Engineer Research and Development Center / Environmental Laboratory. In order to assess if new insensitive, high nitrogen energetics can contribute to eutrophication or increased algal growth in aquatic systems we used the green algae *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*) 96-h growth test performed in basic accordance with U.S. EPA Test Method 1003.0 to assess effects of Triaminoguanidium 1-methyl-5-nitriminotetrazolate (TAGMNT). Significant reductions in algal growth were observed at 0.5 mg/L TAGMNT while a 174% increase in algal growth relative to control was observed at 0.01 mg/L TAGMNT. Ongoing studies to be reported include comparison of TAGMNT responses to current use energetics RDX and TNT.

**MP156 Environmental assessment of depleted uranium used in military armor-piercing rounds in terrestrial systems** J.K. Stanley, U.S. Army Engineer Research and Development Center / Environmental Laboratory, U.S. Army Engineer Research and Development Center / Environmental Laboratory, U.S. Army Engineer Research and Development Center / CEERD-EP-R; J.G. Coleman, US Army Engineer Research and Development Center, US Research and Development Center / Environmental Laboratory, US Army Engineer Research and Development Center / Research Biologist, US Research and Development Center / Research Biologist; S. Brasfield, A.J. Bednar, C.Y. Ang, U.S. Army Engineer Research and Development Center / Environmental Laboratory. Depleted uranium (DU) from the military testing and use of armor-piercing "penetrators" has been shown to accumulate in both soils and sediments; however, little is known about the environmental fate and effects of DU. The purpose of the current study was to assess the bioaccumulation potential and toxic effects of various DU geochemical species to a model terrestrial invertebrate, the earthworm *Eisenia fetida*. This work is part of a larger research focus area to determine the environmental impact of DU munitions and residues in complex active range environments and to develop methods for the cost effective and environmentally protective management and/or removal of small metallic DU and DU residues from affected soils and sands. We will present our research on the assessment of the bioaccumulation of DU in different soil types as well as the effects of DU to earthworm survival, growth, reproduction, behavior (soil avoidance), and cellular stress through soil spiking studies that utilize field collected soils from Yuma and Aberdeen Proving Grounds. Results to date have indicated concentration-dependent bioaccumulation of DU with a biota-to-soil accumulation factor (BSAF) of 0.12; however, no significant reductions in earthworm survivorship or impacts to earthworm cellular stress determined through neutral red retention time (NRRT) assays at soil concentrations of DU up to 1,290 mg DU/kg have been observed. Avoidance of soils contaminated with DU has not been observed in 48-h soil avoidance studies.

**MP157 Phytotoxicity Effects and Uptake of Munitions-Derived Depleted Uranium by Four Grass Species** A.D. Butler, M. Wynter, A.J. Bednar, US Army Engineer Research and Development Center / Environmental Laboratory. Abstract Depleted uranium (DU) contamination is present at Department of Defense installations, primarily as a result of military training, testing, and deployment. Uranium is a naturally occurring element that is typically detected at low concentrations. Munitions-derived DU results from the exposure of DU penetrator rods to the soil and weather, leading to soil contaminated with DU oxides and salts. Past research has shown that DU has relatively low phytotoxicity and that specific chemical forms greatly affect its availability to plants. This study focuses on understanding the effect of DU on plant growth and the environmental fate and transport of weathered DU in plants. A phytotoxicity study is currently being conducted to address low to high soil concentrations contaminated with DU. Seeds of four grass species (*Aristida purpurea* (purple threawn), *Cynodon dactylon* (bermuda grass), *Poa pratensis* (Kentucky bluegrass), and *Panicum virgatum* (switchgrass)) were planted in clean (non-contaminated) and DU-spiked soils at nominal concentrations of 30, 100, 1000 and 3,000 mg/kg. These phytotoxicity tests are being conducted using methods specified in the ASTM standard guide E 1963-98, but slightly modified to evaluate the toxic effect of DU on plant germination, growth, and appearance. Both short ( $\leq 14$  days) and long term ( $\geq 28$  days) tests are being conducted to assess acute and chronic toxicity of DU to plants. At the termination of the test,

phytotoxicity endpoints such as germination percentages, root and shoot lengths, and total plant biomass will be assessed. In addition, plant uptake of DU will be assessed after 28 days. Plant tissues and soils will be analyzed to evaluate the ability of the plants to remove DU from the soil. These results will be presented at the conference.

**MP158 Application of passive sampling devices for underwater unexploded ordnance exposure assessment** G. Rosen, SPAWAR Systems Center Pacific / Environmental, SPAWAR Systems Center Pacific / Scientist, SPAWAR Systems Center / Scientist; W. Wild, SPAWAR Systems Center; G. Lotufo, US Army Engineer Research and Development Center / Environmental Laboratory; J.B. Belden, Oklahoma State University / Department of Zoology. Munitions constituent (MC) exposure to marine biota from potentially leaking unexploded ordnance (UXO) is likely localized and episodic in nature. Thus, passive sampling devices (PSDs) are potentially valuable tools for assessment of MC exposure in the marine environment. The use, and optimization, of commercially available PSDs for MC characterization in marine/estuarine sediments and surface waters was explored. Equilibrium passive sampling involving solid phase microextraction (SPME) fibers was evaluated for estimation of sediment porewater MC concentrations, while polar organic chemical integrative samplers (POCIS) were used for determination of time-weighted average water column concentrations. The approach involved experiments investigating optimal sorbent identification, sampler calibration, and development of sampler-specific uptake kinetics of MC, including TNT, primary TNT degradation products, and RDX and HMX. Subsequent experiments will demonstrate the use of both types of samplers in mesocosm experiments, and final validation of the technologies at underwater UXO sites. Initial results suggest that polyacrilate and divinylbenzene/carboxen/polydimethylsiloxane SPME fiber coatings are optimal for quantification of TNT degradation products (e.g. ADNTs) and RDX, respectively, while hydrophilic-lipophilic balance (HLB) sorbent appears optimal for POCIS samplers for all of the chemicals evaluated. Exposure assessment and management of underwater munitions at sites adjacent to former or active military firing ranges and sites where munitions were previously discarded is of increasing regulatory concern. The successful demonstration and availability of the use of these tools should greatly enhance the ability to conduct exposure assessments of MC at such sites.

**MP159 Quantitative determination of fluorotelomer sulfonates in groundwater and soils around aqueous film-forming foam (AFFF) discharge sites** J.P. Benskin, M.B. Woudneh, R. Grace, J.R. Cosgrove, AXYS Analytical Services Ltd. Fluorotelomer sulfonates (FTSs) have been recently identified as contaminants of groundwater at aqueous film-forming foam (AFFF) discharge sites such as military bases and airports. These substances are a concern due to their tendency to biodegrade to perfluoroalkyl carboxylic acids (PFCAs), which are persistent and ubiquitous contaminants of the global environment. To support monitoring and remediation of AFFF-contaminated sites, we developed methods utilizing solid-phase extraction and liquid chromatography tandem mass spectrometry (HPLC-MS/MS) for analysis of 4:2, 6:2, and 8:2 FTSs in groundwater and soils. Despite the structural similarities between FTSs and perfluoroalkyl sulfonates (PFSA), the former substances are considerably more challenging to monitor by LC-MS/MS due to their tendency to undergo significant matrix-induced ionization enhancement ( $>200\%$  in water and soil depending on the quantity of sample and analytical method). Isotopically-labeled internal standards are not currently available for FTSs, making it difficult to account for such matrix effects. Furthermore, the characteristics of samples obtained from around AFFF sites (e.g. high surfactant concentrations) can result in inconsistencies during sub-sampling and analysis. Here we present new 'matrix effect-free' analytical methods and reliable sampling protocols for accurate and reproducible quantification of FTSs in water and soil. The methods are suitable for up to 100mL of water for 4:2, 6:2 and 8:2 FTS, up to 1g of soil for 4:2 FTS, and up to 5g soil for 6:2 and 8:2 FTS. Detection limits are approximately 5 ng/L in water and 0.2-1.0 ng/g in soil. Percent recoveries ranged from 81-130% and 95-120% for all three FTS congeners in water and soil, respectively. As a further validation of these methods, samples of water and soil from AFFF sites were analyzed for FTSs, PFSA, and PFCAs. The dominant FTS congener in groundwater (6:2 FTS) was significantly correlated with short-chain PFCAs, possibly indicating biodegradation of this substance. These methods represent accurate and quantitative tools to support environmental monitoring and remediation efforts at AFFF-contaminated sites.



**MP160 Human Health Risk Assessment of Hydraulic Fracturing** A. Pawlisz, Conestoga-Rovers & Associates. Increased application of hydraulic fracturing (i.e., fracking) technology, where water, friction reducers, proppants, disinfectants, surfactants, thickeners, scale inhibitors, corrosion inhibitors, and acids are injected into deep shale formations to extract natural gas, has increased calls from the regulatory community and the public for a closer scrutiny of this activity to ensure that fracking is environmentally safe. The growth in fracking has been unprecedented as vast new shale gas plays are discovered and explored across the world. The tremendous expansion in fracking exploration, and the commensurate increase in groundwater extraction/deep chemical injection, have raised concerns over potential health risks to workers, surrounding communities, and the environment posed by impacts to groundwater and surface water, as well as air emissions. This presentation is devoted to exploring the major issues that human health risk assessors are faced with when evaluating the environmental safety of hydraulic fracturing operations.

**MP161 Inferring absence of taxa using fixed-count subsamples of aquatic invertebrates: uncertainty in the US EPA field-based benchmark for conductivity** S.A. Roark, GEI Consultants; C.F. Wolf, G.D. De Jong, GEI Consultants, Inc. / Ecological Division; B.W. Gensemer, GEI Consultants / Ecological Division; S.P. Canton, GEI Consultants, Inc. / Ecological Division. This analysis was conducted to evaluate uncertainty in the US EPA field-based aquatic life benchmark for conductivity in streams in the Appalachian Region as a consequence of inferring absence of taxa using fixed-count subsamples to identify invertebrates in field-collected samples. Subsampling is a widely accepted method used in biomonitoring studies, but has the potential to underestimate true taxa richness, particularly for less common taxa, because taxa may be missed in subsampling. To develop the conductivity benchmark, US EPA inferred "extirpation" of genera from field sites based on absence from subsamples of field-collected benthic community samples. The "extirpation concentration" (XC<sub>95</sub>) for each genus was determined as the 95<sup>th</sup> centile of the weighted cumulative distribution function of conductivity values where the genus was present. The final benchmark value was the 5<sup>th</sup> centile of the ranked genus XC<sub>95</sub>s. The invertebrate community data were collected by the West Virginia Department of Environmental Protection (WVDEP) for multimetric index calculation, and were based on identification of random subsamples of 200 ± 40 individuals from kicknet or dipnet samples. We obtained the WVDEP macroinvertebrate data and filtered it using similar criteria to those used by US EPA. We used resampling without replacement to simulate subsampling and evaluate the influence of subsampling on the inference of absence of taxa. We also determined how the variation in presence/absence conclusion in turn influences the XC<sub>95</sub> calculation. Our results demonstrate that as the subsample size is reduced the number of sites at which a taxon is considered absent increases. In addition, the variability of the XC<sub>95</sub> estimate increases substantially as subsample size decreases, suggesting that erroneous presence/absence determination due to random sampling variation in each subsample results in 2-fold or greater uncertainty in the estimated XC<sub>95</sub> for less common taxa. The combined uncertainty of these XC<sub>95</sub> values translates directly into uncertainty in the conductivity benchmark.

**MP162 Selenium concentrations and solid state chemical speciation in black shale associated with a Kentucky coal seam** J.M. Unrine, University of Kentucky, University of Kentucky / Department of Plant and Soil Sciences, University of Kentucky / Department of Plant & Soil Sciences, University of Kentucky / Savannah River Ecology Laboratory, University of Kentucky / Department of Plant and Soil Sciences, University of Georgia / Savannah River Ecology Laboratory; B. Collin, R. Warner, University of Kentucky. Selenium has emerged as a contaminant of concern within the Appalachian region of the United States due to its potential for release from coal mining sites as well as its potential to elicit adverse population level effects in oviparous vertebrates including birds, fish and amphibians. In order to better understand the weathering and subsequent biogeochemical cycling of Se released from mine waste, we investigated the concentrations and chemical speciation of Se in organic matter rich shale. These shales, often referred to as black shale, comprise a major component of coal mining waste in Appalachia. We selected samples with the highest Se concentrations to analyze using a synchrotron based X-ray microprobe at beamline X26A, National Synchrotron Light Source, Brookhaven National Laboratory. The samples were thin section and mounted in the X-ray beam, which was focused to a spot size of about 5 x 7 µm<sup>2</sup>. A 4-detector element silicon drift

detector was used to measure X-ray fluorescence of Se and other elements contained within the sample. Foci of elevated Se concentrations were then further analyzed using X-ray absorption near edge structure spectroscopy (XANES). We observed localized areas of elevated Se concentrations that were several tens of µm in diameter. The spatial distribution of Se was not consistently associated with the spatial distribution of other elements in the sample, such as Fe. Detection of S in the samples was difficult given that the X-ray optics and detectors were optimized for a higher energy range, above the K 1s electron binding energy for Se (12,658 eV). To determine the Se speciation, the XANES spectra were compared to a standard Se (0) foil as well as selenomethionine (Se -II), sodium selenate (Se +VI) and sodium selenite (Se +IV). The areas of elevated Se intensity appeared to be comprised of primarily elemental Se (0). This indicates that oxidation will be a limiting reaction for the release of Se from these mine wastes. Hence, we hypothesize that source isolation, material handling techniques that limit exposure of black shale strata to oxygen should help to minimize release of Se to the environment and therefore may prove to be a cost-effective control method.

**MP163 Sensitivity comparisons of the mayfly *Centroptilum triangulifer* using reference toxicants and TDS in laboratory and mesocosm exposures** B. Johnson, U.S. EPA / Ecosystems Research Branch; K. Hammer, The McConnell Group c/o U.S. EPA Cincinnati; P. Weaver, The McConnell Group, c/o U.S. EPA; J.M. Lazorchak, U.S. EPA, US Environmental Protection Agency / Office of Research and Development, U.S. EPA Office of Research and Development / National Exposure Research Laboratory, U.S. EPA / Molecular Indicators Research Branch, U.S. EPA / Molecular Ecology Research Branch; J. Shann, University of Cincinnati / Department of Biology; S. Decelles, The McConnell Group c/o U.S. EPA; C. Nietch, U.S. EPA, Office of Research and Development, National Risk Management Laboratory / Water Supply Water Resources Division, U.S. Environmental Protection Agency / Water Supply Water Resources Division, U.S. EPA / Water Supply Water Resources Div; D. Macke, U.S. EPA ORD; J. Allen, U.S. EPA / Office of Research and Development, U.S. EPA / ORD; K. Patnode, U.S. Fish and Wildlife Service c/o U.S. EPA; D. Funk, Stroud Water Research Center. The US EPA has developed a laboratory culture method and toxicity testing procedures for the parthenogenetic mayfly *Centroptilum triangulifer*. We measured growth and mortality of *C. triangulifer* alongside *Ceriodaphnia dubia* and *Daphnia magna* after acute and chronic exposures to standard reference toxicants (NaCl, KCl and CuSO<sub>4</sub>) in the laboratory. We further measured *C. triangulifer* growth and survivorship across a gradient of salt (mixed NaCl and CaCl<sub>2</sub>) concentrations in eight flow-through mesocosms and also compared responses to larval fathead minnows and two mussel species, adultRainbow (*Villosa iris*), juvenile Green floater (*Lasmigona subviridis*). Laboratory studies indicated *C. triangulifer* weight was at least 36% more sensitive to toxicants than either body length or head capsule width. In acute tests, average 50% Lethal Concentrations were 659 mg/L (NaCl), 1957 mg/L (KCl), and 11 µg/L (CuSO<sub>4</sub>). In chronic tests, average 25 % Inhibition Concentrations were 291 mg/L (NaCl), 356 mg/L (KCl) and 6 µg/L (CuSO<sub>4</sub>). Compared to tests with *Ceriodaphnia dubia* and *Daphnia magna*, *C. triangulifer* was the most sensitive species in all NaCl and CuSO<sub>4</sub> tests. For KCl chronic tests the mayfly was equally or more sensitive than *C. dubia* and *D. magna* based on growth rate. Twenty day mesocosm results indicated significantly reduced growth of *C. triangulifer* at all salt concentrations above control (specific conductance: 359 (control) >567=807=1133>1512=2910>4937>8884 µS cm<sup>-1</sup>). Adult emergence occurred only in the control chamber (58% of individuals). Significant mortality occurred at concentrations > 2910 µS cm<sup>-1</sup> with 100% mortality at the highest salt concentration. Calculated median lethal concentration (LC<sub>50</sub>) was 2866 µS cm<sup>-1</sup>. These results indicate the mayfly was more sensitive to TDS in mesocosm exposures than larval fatheads or adult mussels. Juvenile mussels 48-hr LC50 in bench scale tests were 2006.5 mg/L TDS. Further exposure testing of *C. triangulifer* will continue in effort to develop more representative model organisms for assessing impairment to stream populations.

**MP164 Use of Decision Analysis in Regulatory Decision Making** L. Linkov, US Army Corps of Engineers / Engineer Research and Development Center, US Army Engineer Research and Development Center; C. Foran, US Army Corps of Engineers / Engineer Research and Development Center, Army Engineer Research and Development Center / Environmental Laboratory; Z. Collier, US Army Corps of Engineers / Engineer Research & Development Center. Across agencies, regulators responsible for making

environmental permitting decisions for resource extraction or utilization must consider multiple sources and types of information that span the spectrum of technical information and societal pressure. This information is both quantitative and qualitative, and contains uncertainty. Decision making in this type of environment is highly complex and making well-informed value trade-offs is difficult due to the limited rational abilities of humans. Well-documented studies of heuristics and biases show that often decision makers unintentionally skew the decision. Without decision support of some type, regulators run the risk of making sub-optimal decisions. The tools in the field of Decision Analysis can help environmental regulators and permittees make better decisions by structuring the decision making process and allowing for the integration of multiple, dissimilar criteria in which to evaluate alternative courses of action. In this presentation, we review how different permitting decisions are made in several government agencies with regard to natural resource exploitation. By investigating their decision making processes, we can identify strengths and weaknesses and recommend tools and best practices for making regulatory decisions.

**MP165 Brominated flame-retardants in South Africa: burdens in inland and coastal sediments of KwaZulu-Natal Province** M.J. La Guardia,

Virginia Institute of Marine Science / Environmental & Aquatic Animal Health, Virginia Institute of Marine Science / Environmental Science; R.C. Hale, Virginia Institute of Marine Science / Environmental & Aquatic Animal Health, Virginia Institute of Marine Science / Environmental Science Department; B. Newman, Natural Resources and the Environment / Coastal Systems Research Group. Little is known about the usage, production and environmental release of brominated flame retardants (BFRs) in the Southern Hemisphere. Indeed, with the exception of Australia and New Zealand, use and environmental burdens there are either ignored or assumed negligible. An exception has been the interest in long-range atmospheric transport to the Antarctic. To better understand the current state of South Hemispheric BFR usage, release and environmental dissemination, inland and coastal sediments were collected from KwaZulu-Natal Province, South Africa (pop. 3.5 million). Sediments ( $n=44$ ) were analyzed for polybrominated diphenyl ethers (PBDEs), hexabromocyclododecane (HBCD) and alternative-BFRs (alt-BFRs) (2-ethylhexyl 2,3,4,5-tetrabromobenzoate (TBB), 2-ethylhexyl 2,3,4,5-tetrabromophthalate (TBPH), 1,2-bis (2,4,6-tribromophenoxy) ethane (BTBPE) and decabromodiphenyl ethane (DBDPE)) by liquid chromatography-tandem mass spectrometry. BFRs were detected in every sample.  $\Sigma$ PBDEs,  $\Sigma$ HBCD and  $\Sigma$  alt-BFRs ( $\Sigma$ BFR) ranged from 114 to 47,000 ng  $g^{-1}$ , total organic carbon normalized. Median concentrations:  $\Sigma$ PBDEs 1120 ng  $g^{-1}$ ,  $\Sigma$ HBCD 293 ng  $g^{-1}$  and alt-BFRs 364 ng  $g^{-1}$ . The highest sediment concentration ( $\Sigma$ BFR) was detected in Durban Bay (Africa's busiest port) at the mouth of a storm water culvert that drains a large urbanized area. The dominant PBDE congener was BDE-209, 24,500 ng  $g^{-1}$ ; interestingly,  $\Sigma$ HBCD was also high, 21,100 ng  $g^{-1}$ . The sediment also contained TBB, TBPH and DBDPE at 284, 142 and 88 ng  $g^{-1}$ , respectively. The second most contaminated sediment ( $\Sigma$ BFRs 23,100 ng  $g^{-1}$ ) was also collected from the Bay, below the confluence of the Umbilo and Umhlathuzana Rivers. These rivers drain large urbanized areas with many wastewater treatment works, most applying only primary treatment. This sample was dominated by TBB (13,900 ng  $g^{-1}$ ) and congeners of the penta-BDE product, BDE-47 and -99 (3790 and 3050 ng  $g^{-1}$ , respectively). The third most contaminated sediment ( $\Sigma$ BFR 22,200 ng  $g^{-1}$ ) was collected at a storm water drainage ditch located near automotive and plastic manufacturers. The dominant BFR was BDE-209, at 15,500 ng  $g^{-1}$ . Hence, we conclude that BFRs are locally used and released to the South African environment via diverse sources (i.e. runoff, wastewater treatment and manufacturing). This underlines the need to investigate the environmental burdens and risks associated with BFR use in countries of the Southern Hemisphere.

**MP166 Polybrominated Diphenyl Ether (PBDE) Concentrations in Columbia River Sediment and Fish Compared to other Areas in North America** K. Robrock, C. Cushing, M. Kierski, Exponent. Polybrominated diphenyl ethers (PBDEs) are a class of flame retardants that have been linked with neurotoxicity and endocrine disruption effects in mammals and fish.

Increasing PBDE concentrations have been detected in mountain whitefish (*Prosopium williamsoni*), rainbow trout (*Oncorhynchus mykiss*), and sediments in the Columbia River in the region around Trail, British Columbia, Canada, from 1992 to 2005 as part of a biomonitoring program conducted by the Columbia River Integrated Monitoring Program (CRIEMP)—a consortium of Canadian governmental agencies and industries. To understand

how the PBDE concentrations compare with other areas, sediment, mountain whitefish, and rainbow trout PBDE data from the Columbia River in the vicinity of Trail were compared with PBDE data for the same media in rivers and lakes throughout North America, and in particular the Pacific Northwest. A time-trend analysis of these data was also performed. PBDE concentrations rose exponentially in the Columbia River from 1992 to 2004, which was similar to the rates increases reported for fish in the Great Lakes region in the 1990s and early 2000s. PBDE concentrations in Columbia River mountain whitefish collected in 2005 decreased from levels in 2004, similar to trends observed in fish in other parts of North America in the mid to late 2000s. PBDE concentrations in mountain whitefish and rainbow trout from the Trail region collected in 2004 are at the upper end of the concentration range observed elsewhere in the Pacific Northwest during this time (i.e., mid-2000s). Sediment concentrations in the Trail region are also among the highest observed in the Pacific Northwest. It was hypothesized that PBDE concentrations (i.e., both sediment and fish) increase with increasing urban land use near a water body. To investigate this hypothesis, we compared PBDE concentrations from each water body to the population density in the nearest population center upstream of the sample locations. Fish and sediment PBDE concentrations in the rural region around Trail (population approximately 14,000) were higher than concentrations observed in urban areas with higher population densities, indicating that population density is not, in this case, a good predictor for PBDE concentrations in adjacent water bodies.

**MP167 Accumulation of Organohalogen Compounds in Wild Terrestrial Mammals, Raccoon Dog and Masked Palm Civet, from Japan** T. Isobe, Ehime University / Senior Research Fellow Center, Ehime University / Senior Research Fellow, Ehime University / Center for Marine Environmental Studies; M. Yamamoto, Ehime University / Center for Marine Environmental Studies; K. Nomiyama, Ehime University / Center for Marine Environmental Studies (CMES); T. Hayashi, Tochigi Prefectural Museum; S. Yachimori, Shikoku Institute of Natural History; S. Tanabe, Ehime University / Center for Marine Environmental Studies. Information on contamination status and accumulation features of organohalogen compounds, especially of brominated flame retardants (BFRs), in the terrestrial ecosystems is still limited in Japan. In this regard, this study analyzed PCBs (polychlorinated biphenyls), DDTs (dichlorodiphenyltrichloroethane and metabolites), CHLs (chlordane and related compounds), HCHs (hexachlorocyclohexanes), HCB (hexachlorobenzene), PBDEs (polybrominated diphenyl ethers) and HBCDs (hexabromocyclododecanes) in wild terrestrial mammals from several regions of Japan to reveal their contamination status.

Raccoon dogs (*Nyctereutes procyonoides*) and masked palm civets (*Paguma larvata*), which were killed in a traffic accident, were collected from Kochi, Ehime, Osaka, Kanagawa and Tochigi prefectures during 2005-2010. Tissues were lyophilized and extracted with hexane/acetone solution using a high-speed solvent extractor. PCBs and PBDEs were determined using a GC-MS, DDTs, CHLs, HCHs and HCB were measured using a GC-ECD and HBCDs were quantified using a LC-MS/MS. Organohalogen compounds were detected in all the samples analyzed in this study, indicating ubiquitous contamination of Japanese terrestrial ecosystems. CHLs were the dominant compounds, followed by PCBs > PBDEs > DDTs > HCHs > HCB > HBCDs in raccoon dogs. Significant differences in the concentrations of organochlorines in raccoon dogs were observed among sampling locations, but not for PBDEs and HBCDs. This could be due to region-specific usage of organochlorine pesticides in the past. In addition, contributions of hepta-, octa- and nona-BDEs to total PBDEs in raccoon dogs from Kanagawa were higher than those from the other regions, indicating that raccoon dogs from Kanagawa were exposed to BDE congeners originating from technical octa-BDE mixture. On the other hand, DDTs were the predominant compounds, followed by PCBs > CHLs > PBDEs > HCHs > HCB > HBCDs in masked palm civet. Masked palm civet had higher concentrations of PCBs than raccoon dogs in the liver. This may reflect the difference in the metabolic capacity because these two species have similar habitats and feeding habits.

**MP168 Occurrence of Organophosphate Flame Retardants in Environmental Waters and Soils: Three Case Studies in China** J. Liu, X. Wang, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences / State Key Laboratory of Environmental Chemistry and Ecotoxicology. Organophosphate flame retardants (OPEs) are widely used in chemicals, textiles, building materials and electronics industries. Although



the production of OPEs in China was over 70000 tons in 2007, studies on the occurrence in the environment in China are extremely scarce. The contamination of the OPE production process to the surrounding environment was assessed by testing the occurrence of OPEs in the surface water, groundwater and soil samples surrounding a medium-sized OPEs production factory. It was found that all the three OPEs (TEP, TCEP, TCPP) produced in this plant are prevalent in the environmental samples, and the OPE concentrations decreased with the increasing distance of the sampling site from the factory. Up to 2600 ng/g of TEP was detected in the soil samples near the factory, whereas the TEP concentration reduced to 100 ng/g in soil samples collected 3 km away from the plant. Therefore, the intentional or unintentional emissions from the factory production process are the main source of OPEs pollution. The occurrences of OPEs in each step of activated sludge wastewater treatment process and reclaimed water treatment process were studied. The results showed that hydrophilic OPEs including TMP, TEP, TCEP, TCPP and TnBP were dominated in the Beijing Qinghe sewage treatment plant. The OPE concentrations were in the range of tens to hundreds of ng/L, about an order of magnitude lower than that in European sewage treatment plants. Activated sludge treatment process showed good treatment capabilities toward short chain alkanes OPEs but poor treatment capabilities to chlorinated OPEs. In the reclaimed water treatment process, the ultrafiltration, ozone and chlorine method showed very limited capabilities in the removal of OPEs in water. It was observed that OPEs in the effluent were higher than in the influent, which might be attributed to the release of OPEs from materials and piping used in the sewage treatment. The occurrence of OPEs in activities. OPEs in this wetlands come mainly from the source water, human activities and atmospheric transmission. The hydrophilic OPEs like TMP, TEP, TCEP, TCPP and TnBP were dominated in this wetlands. In the area with more frequent human activities, the TEP (110-370 ng/L) and TnBP (6.205 ng/L) concentrations are higher than that in areas with little human activities.

**MP169 Impact of an invasive species (round goby) on the trophic transfer of Montreal wastewater contaminants (PBDEs)** M. Desrosiers, CEAEQ / Ecotoxicologist, CEAEQ / MDDEP / Ecotoxicologie et de l'évaluation du Risque; M. Pelletier, A. Armellin, Environment Canada; Y. Paradis, MRNF. The round goby (*Neogobius melanostomus*), a species from Europe that have recently invaded the Great Lakes and St. Lawrence River, is now the main prey of many predatory fish. This invasive species is also known for its tolerance to pollution, which allows it to colonize areas affected by water of lower quality, such as municipal wastewater. Benthic species with low mobility, the round goby may be exposed to and bioaccumulate more contaminants. The arrival of the round goby in the St. Lawrence may alter the dynamics of contaminant transfer to its predators. The main objective of this project is to evaluate the role of the round goby in the trophic transfer of contaminants. A sampling of sediment, suspended matter, macroinvertebrate and fish was conducted in summer 2010, targeting a dozen sampling stations under the influence of the effluent plume from the sewage plant wastewater from the city of Montreal as well as some control stations outside of this area. We present the results of the concentrations of PBDEs in sediments, suspended solids, and relationships with body burdens of benthic macroinvertebrates, the round goby and other fish-native prey (yellow perch, white sucker) and in piscivorous species such as walleye or bass.

**MP170 Tissue concentrations of Decabrominated diphenyl ether in Chinese Sturgeon (*Acipenser sinensis*)** K. Zhang, University of Alberta; J. HU, Peking University; Y. Wan, Peking University / Toxicology Centre; J.W. Martin, University of Alberta, University of Alberta / Laboratory Medicine and Pathology, University of Alberta / Department of Lab Medicine and Pathology, University of Alberta / Department of Laboratory Medicine and Pathology, Division of Analytical and Environmental Chemistry, University of Alberta / Department of Public Health Science. Penta-BDE and octa-BDE mixtures have been nominated for global ban and phase-out under the Stockholm Convention while deca-BDE continues to be widely used, thus attention has now shifted to deca-BDE mixture. The congener decabromodiphenyl ether (BDE 209) is the primary component in the deca-BDE mixture. Because this compound has a high Log Kow and a relatively high molecular weight, it has been suggested that BDE 209 has very low bioavailability. Furthermore, BDE209 is clarified to be easily metabolized in vitro using fish microsome, and thus was thought not to accumulate in organisms. However, recently, more and more researchs have reported

the high concentrations of BDE209 in fish, birds, grizzly bears, and other animals, demonstrating that BDE209 can enter the biological systems. In the aqueous food web of Lake Winnipeg, which consisted of six species of fish, zooplankton and mussels, BDE209 were found to be biomagnified with TMF of 10.3, which is higher than that of BDE47. Although there are increasing evidences that BDE209 can accumulate in biota, the causes for its bioaccumulation still remain controversial. Therefore, in this study, we study the tissue distribution of BDE209 measure its concentrations in 12 organs and eggs of 17 female Chinese sturgeon (*Acipenser sinensis*). The highest concentrations of BDE209 ( $0.083 \pm 0.12 \mu\text{g/g ww}$ ) were observed in intestine followed by liver ( $0.064 \pm 0.10 \mu\text{g/g ww}$ ) and gills ( $0.056 \pm 0.043 \mu\text{g/g ww}$ ). The concentrations of BDE209 were low in roe and adipose (N.D. to  $0.0021 \pm 0.0039 \mu\text{g/g ww}$ ) although with a relatively high Kow, high distribution to adipose was expected. Extremely different from the less highly substituted PBDEs, lipid-normalization revealed preferential accumulation of PBDEs in muscle ( $5.8 \pm 11.8 \mu\text{g/g ww}$ ), stomach ( $3.9 \pm 3.7 \mu\text{g/g ww}$ ) and intestine ( $3.0 \pm 2.6 \mu\text{g/g ww}$ ). Assisted by the in vitro hepatic metabolism experiment using microsome of Chinese sturgeon, we found that BDE209 can be easily metabolized in Chinese sturgeon. A significant negative relationship was observed between logKow of PBDE congeners and their concentration ratios between heart and intestine, which suggest their transportability in body decrease with log Kow. Such low transportability and the high metabolic capacity may be due to distinct tissue distribution of BDE209 compared with low brominated PBDEs and its bioaccumulation.

**MP171 Phosphorus Flame Retardants in House Dust from E-waste Recycling and Rural Areas in Vietnam** J. Kim, Ehime University / Center for Marine Environmental Studies; T. Isobe, Ehime University / Senior Research Fellow Center, Ehime University / Senior Research Fellow, Ehime University / Center for Marine Environmental Studies; N. Tue, Ehime University / Center for Marine Environmental Studies; M. Muto, Center for Marine Environmental Studies (CMES) / Ehime University; P. Viet, Hanoi University of Science / Centre for Environmental Technology and Sustainable Development; S. Takahashi, S. Tanabe, Ehime University / Center for Marine Environmental Studies. In recent years, use of organophosphorus flame retardants (PFRs) has been increasing due to the ban on common polybrominated diphenyl ethers (PBDE) mixtures (i.e., Penta-BDE and Octa-BDE). PFRs, such as EHDPP (2-ethylhexyl diphenyl phosphate), TnBP (tri-*n*-butyl phosphate), TCP (tricresyl phosphate), TPpP (triphenyl phosphate), TPhP (triphenyl phosphate), TPrP (tripropyl phosphate), TEHP (tris(2-ethylhexyl) phosphate), TCEP (tri(2-ethylhexyl) phosphate), TDCPP (tris(1,3-dichloro-2-propyl) phosphate) are used as flame retardants, plasticizers, anti-foaming agents, and also as additives in paints, glues, lacquers, and varnishes. Potential sources of human exposure to PFRs include indoor air and dust. However, very few studies have reported the occurrence of PFRs in house dust or human exposure to PFRs through house dust ingestion. In the present study, nine PFRs were determined in house dust samples collected from an electronic waste (e-waste) recycling area (Bui Dau:  $n = 16$ ) and a rural area (Duong Quang:  $n = 14$ ) in Vietnam. Total concentrations of the nine PFRs were in the range of 470–5890 ng/g (median 1730 ng/g) in e-waste recycling area and 13–527 ng/g (median 91 ng/g) in rural area. Levels were significantly higher in Bui Dau than those in Duong Quang ( $p < 0.01$ ). In particular, concentrations of EHDPP, TnBP, TCP, TPhP, and TCEP were higher in house dust samples from Bui Dau than those from Duong Quang, suggesting specific release of these compounds during e-waste recycling processes. Among the PFRs analyzed, TPhP was predominant in all the dust samples, with median values of 1450 ng/g ( $< 1.8$ –5070 ng/g) in Bui Dau and 44 ng/g ( $< 1.8$ –183 ng/g) in Duong Quang, respectively. Estimated daily intake (EDI) of each PFR through house dust ingestion in Vietnam was three to four orders of magnitude lower than the reported reference dose (RfD). However, intake of total PFRs by toddlers was estimated as five times higher than that for adults, suggesting potential risk for toddlers if PFRs are continuously used in household products because of toddler's frequent hand-to-mouth contact and tendency to play on the floors. To our knowledge, this is the first study reporting contamination status of PFRs in Vietnamese house microenvironment.

**MP172 Analysis of Methoxylated and Hydroxylated Polybrominated Diphenyl Ethers in Fish Plasma from Eastern Lake Erie** S. Mackintosh, University at Buffalo / Department of Chemistry; A. Perez-Fuentetaja, The State University of New York College at Buffalo / Department of Biology and Great Lakes Center; G. Pacepavicius, Environment Canada / Aquatic



Ecosystem Protection Research Division, Water Science and Technology Directorate; M. Clapsadl, The State University of New York College at Buffalo / Department of Biology and Great Lakes Center; M. Alae, Environment Canada / Aquatic Ecosystem Protection Research Division, Water Science and Technology Directorate; D. Aga, University at Buffalo / Department of Chemistry. Polybrominated diphenyl ethers (PBDEs) have been produced and used globally in many consumer products (furniture, electronics, etc.) as flame retardants. In animals, these compounds have been shown to have toxic effects on the endocrine system, thyroid function, and early neurodevelopment. In this study, PBDE concentrations in whole body composites from five fish species (steelhead trout, walleye, lake trout, small mouth bass, and yellow perch) were determined. Concentrations of  $\Sigma$ PBDEs (sum of 26 congeners) ranged from 2.42 ng/g wet weight (ww) in yellow perch to 31.44 ng/g ww walleye. Methoxylated-PBDEs (MeO-PBDEs) and hydroxylated-PBDEs (OH-PBDEs) are well known metabolites of PBDEs. These compounds are detected primarily in blood and have been linked to adverse effects due to structural similarity to the thyroid hormone, thyroxine (T4). In assessing the impacts of PBDEs in fish from Lake Erie it is essential to monitor parent PBDE concentrations in plasma in addition to MeO-PBDEs and OH-PBDEs concentrations. The aim of this work is to determine the occurrence of PBDEs, MeO-PBDEs, and OH-PBDEs in plasma from selected fish species from Lake Erie. Concentrations and homologue patterns will be discussed and plasma contaminate loads will be compared to whole fish levels.

**MP173 Organophosphate Flame Retardant Levels in Indoor Dust and Exposure to Toddlers** J. Misenheimer, H.M. Stapleton, Duke University / Nicholas School of the Environment. Flame retardants are a class of contaminants of concern due to their increased use in consumer products as well as research demonstrating associations with negative health outcomes. As polybrominated diphenyl ethers (PBDEs) are being phased out from use, new classes of flame retardants are increasing in production. One specific organophosphate flame retardant (OPFR), tris (1-3-dichloropropyl) phosphate (TDCPP), was historically used in children's pajamas, but was later phased-out after being identified as a mutagen. However, TDCPP and other OPFRs are now commonly used in polyurethane foam, and have been shown to accumulate in indoor (e.g. house dust) and outdoor environments. It is also well known that toddlers receive more exposure to chemicals found in house dust relative to adults due to crawling and mouthing behaviors. The objective of this study was to investigate toddler's exposure to OPFRs in indoor environments through analysis of paired house dust and hand wipe samples collected from young children. Between January and March 2012, our research team re-contacted families with young children who had participated in our previous research study on PBDEs during 2009-2010. A research team visited the home of all consenting families (n=30) to collect paired house dust samples and hand wipe samples from children between the ages of 1-4 years of age. House dust samples were sieved to < 500 microns and both dust and hand wipes were analyzed for a suite of organophosphate flame retardants by gas chromatography mass spectrometry. TDCPP and triphenylphosphate (TPP) were detected in 100% of the dust samples analyzed. Tris (2-chloroethyl) phosphate (TCEP) and tris (chloropropyl) phosphate (TCPP) were detected in 97% of the samples. The geometric mean levels were 344, 3396, 2739, and 213 ng/g for TCEP, TCPP, TDCPP and TPP, respectively. Using EPA's estimate of dust exposure in children (50 mg/day), an average toddler's intake of TDCPP is expected to be 136.96 ng/day. Children in the 95<sup>th</sup> percentile are expected to intake 521.13 ng/day of TDCPP. The sum for ingestion of all four OPFRs was found to be 334.64 ng/day for an average child and 2485.5 ng/day for a child in the upper 95<sup>th</sup> percentile. Analysis of flame retardants in hand wipes is in progress to examine associations with levels measured in dust to better predict exposure to these OPFRs.

**MP174 Characterization of Exposure to Brominated Flame Retardants in Electronic Waste** E. Krupka, G. Molnar, CDM Smith. Brominated flame retardants (BFRs) are added to the plastic housings of electronics for the purpose of fire prevention. They are halogenated compounds which are highly reactive. BFRs work by slowing combustion; as the equipment they are contained in reaches high temperatures, bromine atoms are released which reduce the amount of oxygen available to continue a fire. BFRs are not permanently bound to the polymer matrix; therefore they are capable of leaching out and subsequently threatening human and/or environmental health. Human or ecological exposure can occur through the direct handling

of electronic wastes during recycling, or by direct contact with or ingestion of contaminated soil or water or inhalation of air. Recent regulations have restricted the use of certain BFRs (such as polybrominated biphenyl ethers or PBDEs), but there is limited knowledge of the pathways and effects of exposure to alternative flame retardants, including non-PBDE BFRs. Characterization of these BFRs is essential as the use of alternative flame retardants is important in reducing the toxicity of electronic wastes.

**MP175 Assessment of polybrominated diphenyl ethers and 2,2',4,4',5,5'-hexabromobiphenyl in bottom sediment of some effluent-receiving Rivers in Cape Town** O.S. Fatoki, Cape Peninsula University of Technology / Department of Chemistry; J.P. Odendaal, Cape Peninsula University of Technology / Department of Environmental and Occupational Studies; A.P. Daso, Cape Peninsula University of Technology / Department of Food and Agricultural Sciences. Until recently, studies reporting the environmental levels of polybrominated diphenyl ethers and polybrominated biphenyls in Africa are rarely found in the literature. Consequently, this study was aimed to assess the concentrations of commonly investigated PBDE congeners (BDE 28, 47, 100, 99, 154, 153, 183 and 209) as well as BB 153 in bottom sediment of two rivers receiving effluent discharges within the City of Cape Town. In this study, both GC- $\mu$ ECD and GC-TOF-MS techniques were employed for the routine analysis and the structural elucidation of the target compounds, respectively. The overall mean concentrations of the total PBDE ranged from 0.06 – 2.47, 0.07 – 0.58 and 0.10 – 0.78 ng/g for the upstream, point of discharge and downstream sampling points, respectively. These values were relatively higher in the Kuils River samples where the overall mean concentrations were found to be 0.22 – 1.52, 2.36 – 9.95 and 0.31 – 5.38 ng/g for the upstream, point of discharge and downstream sampling points, respectively. The average levels of BB 153, which were generally low, ranged from ND – 0.33, ND – 0.22 and 0.04 – 0.39 ng/g for the upstream, point of discharge and downstream sampling points, respectively. Similarly, the average levels of BB 153 in the Kuils River were in the range of ND – 0.18, ND – 1.01 and ND – 1.21 ng/g for the upstream, point of discharge and downstream sampling points, respectively. The statistical analyses performed on these data showed that majority of the PBDE congeners, except BDE 209 had strong positive correlation with the total organic carbon (TOC). Furthermore, the Pearson's correlation analyses conducted amongst the congeners showed that majority of the PBDE congeners as well as BB 153 had strong positive relationship with one another, which were statistically significant at both 0.01 and 0.05 (p-values), thus suggesting the possible similarity in the sources of these pollutants into the investigated rivers. This study further confirms the potential of wastewater treatment plants' discharges as important source of PBDEs in the receiving environment.

**MP176 Out with the old in with the new: BFRs in Toronto** E. Goosey, A. Saini, University of Toronto; G. Abbasi, University of Toronto / Department of Geography; L. Melymuk, University of Toronto / Department of Chemical Engineering and Applied Chemistry, University of Toronto / Dept. of Chemical Engineering and Applied Chemistry; M.L. Diamond, University of Toronto / Department of Geography and program in planning, University of Toronto / Department of Geography. One quarter of Canada's population lives in Toronto ([www.toronto.ca](http://www.toronto.ca)). Not surprisingly, the city acts as a major source of flame retardants to Lake Ontario and its aquatic ecosystem (Diamond et al. 2010). Concentrations of polybrominated diphenyl ether (PBDE) in the lake are expected to following declining air concentrations as a result of use restrictions in North America and Europe. However, despite restrictions on PBDE use in Canada, the importation of products containing these compounds is not restricted and, along with a large inventory of goods still in use containing PBDEs, the extent to which these restrictions will impact PBDEs in Lake Ontario is unclear. To complicate the situation, the globalization of commodity production is seeing the widespread adoption the California flammability standard for the flexible foam in furniture, as laid out in California Technical Bulletin TB 117. Penta-BDE had largely been used in flexible foam to achieve this standard. With Penta and Octa-BDEs largely banned from new production and with Deca on the way out, we are seeing a range of "new" flame retardants (NFRs) in indoor and outdoor media. This paper provides results from a 16 month campaign to measure outdoor air concentrations using passive and hi-vol samplers. The passive samplers were deployed along an urban-suburban transect in Toronto. Results from this campaign are compared to past measurements (Melymuk et al. 2012). In addition, passive samplers were

deployed and dust samples taken in 5 homes (14 rooms total) in Toronto. All samples were analyzed for 12 NFR compounds and 15 PBDE congeners. The NFRs highest indoors were ethylhexyl-tetrabromobenzene (TBB) and bis(2-ethylhexyl) tetrabromophthalate (TBPH) at levels comparable to those reported by Stapleton and co-workers for the US, and tris-dichlorophenyl phosphate (TDCPP) at levels much lower than those reported by Stapleton and co-workers. DecaBDE replacements brominated trimethylphenylindane (OBIND) and decabromodiphenylethane (DBDPE) were present in 60% of indoor samples.

**MP177 Development of *in situ* hybridization –based analysis for examining mRNA expression of thyroid regulating deiodinases in Zebrafish (Danio rerio)** W. Dong, Nicholas School of the Environment, Duke University, Durham, NC / Researcher; L. Macaulay, K. Kwok, D. Hinton, H. Stapleton, Nicholas School of the Environment, Duke University, Durham, NC. Although RT-PCR is used widely for gene expression quantification in tissues, it cannot examine localized gene expression; and, isolation of organs from zebrafish embryos is technically difficult. Whole embryo/organism gene expression can often be misleading as expression patterns vary between organs and toxic effects may be masked if expression changes are highly localized in specific tissues. *In situ* hybridization provides an excellent platform to localize and quantify gene expression *in vivo*. In this study we developed an *in situ* hybridization assay to examine mRNA expression of several thyroid regulating genes, including several thyroid hormone deiodinases (Di Types 1 and 2), and several thyroid hormone nuclear receptors (THR $\alpha$  and THR $\beta$ ) at different zebrafish life stages (2 cells, 8 cells, 22 hpf, 24 hpf, 48 hpf, 72 hpf, and adult) and showed life stage specific differential expression and locations of these genes. Since several classes of halogenated organic contaminants are also known to impact thyroid regulation and several of these proteins, we investigated the effects of polybrominated diphenyl ethers (PBDEs), and their hydroxyl-metabolites (OH-PBDEs), on thyroid gene expression using this technique. Zebrafish embryos were exposed to 100 nM of 6-OH-BDE-47 starting at 4 hpf for 24 hours. After exposure, embryos were fixed and expression of Di1 and 2 were examined relative to control embryos. Exposure resulted in a 41% increase ( $p < 0.05$ ) in mean intensity of Di1 expression and a 25% increase ( $p = 0.07$ ) in Di2 expression in midbrains quantified by measuring intensity of coloration using ImageJ analysis software. Future research will examine the effects of 6-OH-BDE 47 on mRNA expression of these genes at different developmental stages. Our results indicate that *in situ* hybridization enables analysis of effects of OH-PBDEs, and possibly other halogenated contaminants, on thyroid genes expression, providing more insight into its toxic mechanism and identifying sites of injury in different life stages.

**MP178 Biodegradation of Halogen-Free Flame Retardants** S.L. Waaijers, University of Amsterdam / Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam/IBED Institute; R. Helmus, C. Blair, J.R. Parsons, M.H. Kraak, W. Admiraal, P. de Voogt, University of Amsterdam. Several halogenated flame retardants have been banned due to their persistence, bioaccumulation potential, toxicity (PBT) and probable environmental risk. Hence, halogen-free flame retardants (HFFR) are required and a range of new flame retardants (FR) have been proposed. However, reviewing the literature we demonstrated that the PBT properties of most alternative flame retardants are poorly characterized. Therefore, the aim of the present study was to generate persistency data of HFFRs by performing biodegradation studies with diluted waste water treatment sludge. To determine the aerobic biodegradability of the selected compounds, we performed a 28 day study based on OECD guideline 301. To monitor degradation each parent compound was analyzed over time using HPLC-MS/MS. The alternative flame retardants studied were three organic phosphates (bisphenol a bis(diphenyl phosphate) (BDP), dihydro oxa phosphaphenanthrene (DOPO) and resorcinol bis(diphenyl phosphate) (RDP)). As a positive control the easily degradable benzoic acid was tested as well. Controls were also set-up to study abiotic degradation and HFFR toxicity to the micro-organisms. In addition to studying primary biodegradation, a selection of potential breakdown products will also be analyzed, such as the toxic compound bisphenol a. Our results will contribute to the evaluation of the environmental safety of these HFFR and their potential as alternatives for the halogenated flame retardants.

**MP179 Present and future of flame retardants in consumer products; An SFA application** G. Abbasi, M. Diamond, University of Toronto / Geography and urban planning; M. Murray, National Wildlife Federation; A. Soehl, Great Lakes Commission. Brominated flame retardants (BFRs) are synthetic additives which are widely used to reduce the flammability in consumer products. Due to their persistent, bioaccumulative and toxic (PBT) properties, some representative BFRs such as polybrominated diphenyl ethers (PBDEs) have been regulated or phased out in Europe and North America. Despite the global decline in the use of PBDEs, flame retarded products will continue to contribute to the migration of these compounds to indoor and then outdoor environments. Further, BFRs may return into consumer products with recycling flame retarded products at the end of their life cycle. In this study, a substance flow analysis (SFA) was performed to characterize the flow of these substances and their replacements from product inventory input. This system is built upon a detailed dynamic inventory of flame retardant products for the City of Toronto. The results show changing BFR levels over time as a result of changes in flame retardant substances in consumer products, use pattern and waste management. The outcome of such a study can serve as a rational basis for planning systematic action and measures to reduce the use of and human exposure to these substances.

**MP180 Active sampling for bioavailable contaminants using SPE as a surrogate for body burden – the *in situ* sampler for bioavailability (IS2B)** S.D. Supowit, Arizona State University / Department of Civil and Environmental Engineering; I.B. Roll, Arizona State University; V.D. DANG, K.J. Kroll, University of Florida / Physiological Sciences; r. halden, Arizona State University / Biodesign Institute; N.D. Denslow, University of Florida / Department of Physiological Sciences and Center for Environmental and Human Toxicology, University of Florida / Dept. of Physiological Sciences &. Contamination of surface water sediments with persistent chemicals like insecticides, pharmaceuticals, and antimicrobials is an ongoing concern because these pollutants can be toxic, endocrine-disrupting, carcinogenic, and bioaccumulative. It is therefore important to determine the bioavailability of these contaminants in their environmental sink, i.e., in aquatic sediments. Direct analysis for bioavailability of environmental contaminants can be cumbersome because it requires the exposure, capture, and subsequent analysis of biota living in contaminated environments to accurately determine body burdens associated with sediment pollution. In the present, ongoing study, we explore the risk posed by sediment-borne contaminants, including fipronil, triclosan, triclocarban, *p,p'*-DDE, and dieldrin. We have designed an active sampling device that includes a chemical surrogate for bioavailability studies in the form of a solid phase extraction (SPE) resin containing hydrophobic ligands, e.g., C-18 or pyrrolidinone. A stainless-steel tube containing a six-channel peristaltic pump is placed into contaminated sediment, and the pump separately delivers sufficient amounts of pore water and bulk water (mL to L) through an array of SPE cartridges, thereby concentrating dissolved chemicals from the aqueous phase that is assumed to be representative of the bioavailable fraction of the total mass of contaminants present. The SPE cartridges act as surrogates for biota in this innovative device. The contaminant mass captured with the SPE resins *in situ* can then be analyzed in the laboratory via GC-MS/MS or LC-MS/MS and compared to the body burden of biota exposed in mesocosms experiments. The intent of the project is to formulate and validate mathematical models that enable one to translate measurements obtained with the *in situ* sampler for bioavailability (IS2B) directly into body burden estimates for relevant aquatic macrobiota, thereby informing environmental risk assessments without the need for sacrificing animals in the process.

**MP181 Assessing Bioavailability of Persistent Organic Pollutants (POPs) in Mesocosms** V.D. DANG, University of Florida / Physiological Sciences; K.J. Kroll, University of Florida; S. Supowit, I.B. Roll, R.U. Halden, Arizona State University; N.D. Denslow, University of Florida. Sediment contaminated with hydrophobic organic pollutants (HOCs) poses potential risks to the aquatic environment and to human health. The mass of contaminants taken up into organisms, i.e., the body burden, is often used to assess bioavailability of HOCs in contaminated sediments. In this research, we are using artificial mesocosms to better understand the bioavailability of hydrophobic pollutants. Control sediments featuring a high total organic carbon content but with no detectable levels of HOCs have been obtained from the muck farms of the North shore of Lake Apopka, FL. These sediments are being used to set up mesocosms spiked with one of five HOCs or a mixture of *p,p'*-DDE, dieldrin, triclosan, triclocarban, and

fipronil. First, we determined the best method to spike HOCs into the sediments, including mixing the sediments with glass beads evenly coated with the contaminant, or dripping contaminants dissolved in an acetonitrile stock solution or from a methanolic stock solution into a slurry of sediments and water agitated by a mixer. Analytical tools utilized in this work include GC/MS and LC/MS to evaluate which spiking method gives the most homogeneous distribution of HOCs and to determine the sorption equilibrium between porewater and sediment. The contaminated sediment serves as a substrate for bioaccumulation of HOCs in blackworms (*Lumbriculus variegatus*). Subsamples of biota collected at different time intervals are used to examine contaminant uptake patterns. These fundamental data are required to provide insight into the *in situ* sampler for bioavailability assessment (IS2B) that has been developed by researchers from Arizona State University. This active sampling device enables collection of contaminants from both porewater and surface water simultaneously.

**MP182 Assessment of performance of carbon amended sand caps using passive sampling of porewater PAH migration** U. Ghosh, University of Maryland Baltimore County / Civil & Environmental Engineering, University of Maryland Baltimore County / Department of Chemical, Biochemical, and Environmental Engineering, University of Maryland Baltimore County / Chemical, Biochemical, and Environmental Engineering; P. Gidley, University of Maryland Baltimore County. Typical sand caps used for sediment remediation have little sorption capacity to retard the migration of hydrophobic contaminants such as PAHs that can be mobilized by significant groundwater flow. Laboratory column experiments were performed using contaminated sediments and capping materials from a creosote contaminated USEPA Superfund site. Azoic laboratory column experiments demonstrated rapid breakthrough of lower molecular weight PAHs when groundwater seepage was simulated through a column packed with coarse sand capping material. After eight pore volumes of flow, most PAHs measured showed at least 50% of initial source pore water concentrations at the surface of 65 cm capping material. PAH concentration in the cap solids was low and comparable to background levels typically seen in urban depositional sediment, but the pore water concentrations were high. Column experiments with a peat amendment delayed PAH breakthrough. The most dramatic result was observed for caps amended with activated carbon at a dose of 2% by dry weight. PAH concentrations in the pore water of the activated carbon amended caps were 3-4 orders of magnitude lower ( $0.04 \pm 0.02$   $\mu\text{g/L}$  for pyrene) than concentrations in the pore water of the source sediments ( $26.2 \pm 5.6$   $\mu\text{g/L}$  for pyrene) even after several hundred pore volumes of flow. Enhancing the sorption capacity of caps with activated carbon amendment even at a lower dose of 0.2% demonstrated a significant impact on contaminant retardation suggesting consideration of active capping for field sites prone to groundwater upwelling or where thin caps are desired to minimize change in bathymetry and impacts to aquatic habitats.

**MP183 Combining synchrotron microprobe and in vitro bioassay to interrogate bioaccessibility of mine tailings** R.A. Root, R. Ruthforth, University of Arizona / Soil, Water and Environmental Science; C. Lantz, University of Arizona / Cell Biology and Anatomy; J. Chorover, University of Arizona / Soil, Water and Environmental Science, University of Arizona / Arizona Laboratory for Emerging Contaminants. Arsenic speciation and in vitro bio-assays on geo-dusts can be combined to give a comprehensive understanding of the bio-accessibility and toxic exposure risk associated with aerosolized contaminants from mine tailings in arid environments. Micro-focused ( $\sim 2\mu\text{m}$  beam spot size) synchrotron x-ray diffraction, absorption, and multiple energy fluorescence can provide information on elemental concentrations and associations, crystal structure, and oxidation state of individual ingestible and inhalable particles ( $< 250\mu\text{m}$  and  $< 10\mu\text{m}$  respectively). This technique can distinguish particles of varying solubility from contaminant host sorbents and arsenic bearing minerals in a complex heterogeneous dust sample matrix. In vitro bio-fluid simulations have been used to estimate the fraction of metals in tailings from the Klondike mine (AZ, USA) that are bioaccessible in human lungs or gastrointestinal track at different time intervals. Comparing 1 hr and 1 week exposures in synthetic lung fluid, the relative release of Al and Pb was 1261% and 400% greater, respectively, for 1 hr than 1 week; while As and Cu relative releases were 265% and 233% greater at 1 week versus 1 hour, showing an elemental dependence on temporal bio-accessibility in alveolar fluid. In the synthetic gastrointestinal fluid Al, As, Cu, and Pb showed relative increased releases of 1680%, 9310%, 9340%, and 8170% versus an aqueous extraction, while

and Ca and Zn had a relative increase of 740% and 7063% in aqueous extracts versus synthetic gastrointestinal fluid. Targeting exposure sites within the human body and interrogating the speciation of contaminants with the combined application of in vitro bioassay and x-ray micro probe techniques will elucidate potential risk to human or ecosystem health from mine tailings derived geo-dusts.

**MP184 Nature or Nurture: Does metal speciation or soil matrix control the bioaccessibility and human health risks of metals in soils?** Y. Shen, Harvard School of Public Health / Department of Environmental Health; E. Estes, Harvard School of Engineering and Applied Sciences / Department of Earth and Planetary Sciences; S. Shdo, J.P. Shine, Harvard School of Public Health / Department of Environmental Health. Heavy metal contamination in soil is ubiquitous due to past and current mining activity, smelting, and other industrial processes. An important pathway of human exposure to those metals is via the oral ingestion of soil. Because the bioaccessible fraction of metals can be highly variable, the use of a single default value for the fraction of total metals that are bioaccessible is ill-advised for risk assessment purposes. To better understand the combined role of solid-phase metal speciation and soil matrix effects on bioaccessibility, seventeen forms of metal minerals that would commonly be at present in soil were obtained from the Mineralogical Museum at Harvard University. As a baseline, metal minerals and salts containing Pb, Zn, As, Cu were spiked into a clean loam soil individually. In-vitro simple bioaccessibility extraction tests (SBET) and a six-step sequential extraction procedure were conducted on the spiked soils (as well as on the pure phase minerals themselves). To further examine the effect of soil matrix on bioaccessibility, the spiked soils were also amended with ferrihydrite (0.5%) or humic acid (10%). The presence of humic acid in soil decreased the bioaccessibility of metal oxides and metal hydroxides to more of an extent than ferrihydrite. Noticeably, the presence of ferrihydrite increased the bioaccessibility of metal sulfides. Corresponding changes of the underlying metal speciation in the soil matrix (particularly iron speciation) were determined with the use of sequential extractions. The results of this study facilitate a more informed discussion on the relative importance of metal mineralogical composition and soil properties on metal bioaccessibility, how to characterize metal bioaccessibility via oral ingestion more precisely, and how to improve the framework of metal bioavailability prediction for regulatory purposes.

**MP185 Nutrition as a new paradigm in detection and remediation of environmental pollutants** B. Newsome, University of Kentucky / Department of Chemistry; D. Bhattacharyya, J.Z. Hilt, T. Dziubla, University of Kentucky / Department of Chemical and Materials Engineering; B. Hennig, University of Kentucky / UK Superfund Research Center. Nutrition research has resulted in discoveries that extend beyond healthy living and diet-related chronic diseases and enter into the realms of risk assessment and remediation. One example of this is in the use of various bio-molecules and antioxidants (e.g. quercetin and other polyphenols), which have been found to bind persistent environmental pollutants and modulate their toxicity. This binding affinity can be utilized for biomimetic approaches to detection and remediation. This research involves two methods for capture, detection and remediation of PCBs and TCE, which have potential as rapid methods for contaminated water and sediment remediation through pollutant removal and detoxification via nontoxic, green approaches. Magnetic iron oxide core nanoparticles (MNPs) were functionalized with multiple surface chemistries, including citric acid, oleic acid, polyphenolic, and polystyrene-based coatings, to test PCB binding capacity. FT-IR and LC/MS were used to analyze affinity and absorption characteristics of PCBs toward these nanocomposites in aqueous systems. Citric acid- and oleic acid-coated Fe NPs show high binding affinities for PCB126 with total PCB binding occurring at 0.1 mg/mL and 2 mg/mL particle concentrations, respectively, in PCB-contaminated water samples. Quercetin multi-acrylate (QMA) polyphenolic NPs have been successfully synthesized with iron oxide cores for magnetic separation from contaminated water samples. QMA enhances PCB126 binding to polymer-coated MNPs more than two-fold; coatings formulated with stimuli-responsive hydrogels (PNIPAAm) allow for binding and release of PCB for reuse of NPs in high-throughput setups. Additionally, QMA-PNIPAAm-MNPs and PCB demonstrate a concentration-dependent fluorescence shift, which allows for pollutant binding and removal as well as for sensing. Fe/Pd bimetallic nanoparticles have been directly synthesized in PNIPAAm and membranes (with poly-acrylic acid) for stable, high throughput reductive decomposition of TCE and PCB. For the oxidative pathway,



Fe is converted to iron oxide NPs and use of  $H_2O_2$  creates  $OH^\bullet$ , which reacts with dissolved TCE/PCB at diffusion-controlled limits;  $Cl^-$  formation confirmed compound oxidation. Polyphenols are also being used for green synthesis of supported Fe and bimetallic Fe/Pd NPs on membranes and hydrogels; polyphenols directly complex and then reduce Fe to zero valent NPs in the matrix thus eliminating loss or aggregation issues.

**MP186 Real-time PAH Immunosensor Development: Immunogen Design and Antibody Selection for Detection of a PAH Spectrum at Remediation Sites** C.R. Spier, University of California, Davis / Entomology; M.A. Unger, Virginia Institute of Marine Science / Environmental and Aquatic Animal Health; S.L. Kaattari, Virginia Institute of Marine Science / Environmental and Aquatic Animal Health, Virginia Institute of Marine Science / Professor. Employment of antibody-based sensors for the quantitative detection of environmental pollutants such as PAHs possess advantages of exquisite sensitivity (sub-ppb), quick turn-around (< 10 minutes), and little or no sample preparation. A limitation to this technology; however, can arise from the relatively low immunogenicity of these small, hydrophobic epitopes. Often the linker arm, by which these analytes are conjugated to an immunogenic carrier, can introduce inappropriate and highly immunogenic structures to the antigen, deflecting recognition from the analyte itself. To improve upon the specificities we have already achieved, and provide for the next generation of high resolution antibodies, we have examined the effect of PAH derivatization and alkylation on antibody specificity. Murine polyclonal antibodies were characterized by competitive ELISA, using the unmodified analytes and their derivatives to determine relative affinity and specificity. Characterization of the antibodies provided insights into the structural constraints necessary to maintain specificity to these relatively non-immunogenic analytes. It was found that 1) suitable hapten size for small hydrophobic molecular recognition should be larger than one aromatic ring (unsubstituted); 2) analyte derivatization must avoid introduction of substituents that disrupt a pre-existing immunogenic feature or provide for a more highly immunogenic feature; 3) antibody specificity can uniquely decrease over time and must be tightly monitored; and 4) heterologous hapten-carriers can, at times, be used to detect the original analyte. To provide for a more differentiative, analytical profile of PAHs to be found within remediation sites, we have translated the above observations into a plan for the production of additional field-deployable antibody reagents.

**MP187 Use of Passive Samplers to Measure Dissolved Organic Contaminants in a Temperate Estuary** M.M. Perron, NRC/EPA; R.M. Burgess, U.S. EPA / Atlantic Ecology Division, National Health and Environmental Effects Research Laboratory, US EPA, U.S. EPA, U.S. Environmental Protection Agency / Ord/Nheerl-Atlantic Ecology Div, U.S. EPA / Ord/Nheerl-Atlantic Ecology Div; M.G. Cantwell, / Atlantic Ecology Division; E.M. Suuberg, Brown University / School of Engineering. Measuring dissolved concentrations of organic contaminants can be challenging given their low solubilities and high particle association. However, to perform accurate risk assessments of these chemicals, knowing the dissolved concentration is critical since it is considered to be the best measure of biological exposure in aquatic systems. Recently, passive sampling methods have been used for measuring dissolved concentrations in water and sediments. To date, the emphasis of passive sampler development and use has been on monitoring legacy contaminants, but the technology is applicable to some emerging contaminants although data for this class of chemicals is currently very limited. In this study, several contaminants were measured in water column deployments using polyethylene (PE) and polyoxymethylene (POM) passive samplers. Samplers were deployed in galvanized cages for 21 days in Narragansett Bay, RI (USA) and solvent extracted upon recovery to analyze for legacy and emerging contaminants. For each contaminant, dissolved concentrations were calculated using measured passive sampler concentrations and sampler-water partition coefficients derived in the laboratory. Differences observed between dissolved concentrations obtained from PE and POM will be presented for polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs) and triclosan. This work will provide useful information for monitoring legacy and emerging contaminants in the water column of a temperate estuary and provide information to environmental managers for selecting types of passive samplers for deployment.

**MP188 Adsorption of selected pharmaceuticals in melanin (sepia officinalis) with detection by LC-MS/MS** A. Lilliehook, Umea University / Dept. of Chemistry; I. Brandt, Uppsala University / Department of Environmental Toxicology; M. Tysklind, University of Umea / Department of Chemistry; J. Fick, Umea University / Department of Chemistry. Traditional environmental toxins like persistent organic pollutants (POP's) which to a degree accumulate in body fat and can stay unchanged under longer times in the environment and in biological materials. Pharmaceuticals behave more irrational due to the wide range of chemical structures and different usages that is gathered under this group. Lipophilic pharmaceuticals ( $pK_a$  over 7 and positive log  $P$ ) do not behave as POP's, there is yet no body compartment introduced that can store these drugs. For this reason, it is difficult to establish chronic or acute dose-response relationships in environmentally exposed fish. The interactions between melanin, the brown/black pigment that is present in many different regions of animals, and different pharmaceuticals have been a subject of interest since 1962 when the first paper on it was released. Various CNS-active drugs, antibiotics, analgesics and local anesthetics are bound to melanin-and can remain in pigmented tissues at time-points when the drugs have been eliminated from plasma and non-pigmented tissues. 40% of all pharmaceuticals are suspected to be able to bind to melanin pigments. It has been shown that pharmaceuticals that are basic ( $pK_a$  over 7) and lipophilic (positive log  $P$ ) bind well to melanin. Melanin pigment can be utilized as an endogenous trapping agent to monitor certain pharmaceuticals in contaminated water, using a highly pigmented fish species as the test organism. In doing so, we take advantage of the fact that the pigment melanin can efficiently trap numerous pharmaceuticals and retain them for long periods of. To test pharmaceuticals binding kinetics to melanin 10 pharmaceuticals are measured (chloroquine, chlorpromazine, ciprofloxacin, haloperidol, memantine, oxazepam, perphenazine, risperidone, sotalol and trimethoprim) in melanin from common cuttlefish (*Sepia Officinalis*) phosphate buffer saline (PBS) mix. Three different parameters have been tested; concentration, time and temperature. At three different temperatures, 5 different concentrations are measured during 6 different times. For the sample treatment of melanin mixture a syringe coupled to a 0.45 mm injection based filter was used to separate melanin fraction from the liquid fraction. The samples triplicates were analyzed on LC- MS/MS. It is possible to observe a trend where some of the above drugs bind to melanin in PBS solution and the ones that bind to melanin seems to do so at a fast rate.

**MP189 Effect of tidal cycle on the total petroleum hydrocarbon concentrations in intertidal seawater after the Hebei Spirit oil spill** M. Kim, Korea Ocean Research and Development Institute / Oil & POPs Research Group; S. Hong, Korea Ocean Research & Development Institute / Oil and POPs research group, Korea Ocean Research and Development Institute / Oil & POPs Research Group; J. Won, Korea Marine Environment Management Corporation / Climate & Marine Environment Team; U. Yim, Korea Ocean Research and Development Institute / Oil & POPs Research Group, Korea Ocean Research & Development Institute; S. Ha, J. An, G. Han, J. Jung, W. Shim, Korea Ocean Research and Development Institute / Oil & POPs Research Group.

**MP190 Mesocosm Study on Weathering Characteristics of Iranian Heavy Crude Oil with and without Dispersants** C. Joo, Korea Ocean Research and Development Institute / Oil and POPs research group; W. Shim, U. Yim, S. Ha, M. Kim, J. An, E. Kim, Y. Kim, Korea Ocean Research and Development Institute; G. Kim, Gyeongsang National University. The environmental fate of Iranian Heavy [ME1] crude oil (IHC) with and without an added oil spill dispersant (OSD) has been studied using a 1,000 kL capacity *in situ* mesocosm. Physical weathering and chemical composition changes of the oil were monitored for 77 days. The OSD significantly changed the chemical fingerprint of the oil. Compound-specific effects of the OSD could be observed as changes over time in the content of the TPH, unresolved complex mixture (UCM), alkanes, PAHs, hopanes and steranes in the oil. When oil weathers, most hydrocarbons show a rapidly decreasing phase followed by a slowdown and stabilization. Recalcitrant biomarkers, however, show an opposite trend. An additional ca. 10% increase in hydrocarbon formation in the form of UCM occurred after OSD treatment. This [ME2] change, in effect, increased the half-life of the TPH in the oil with the dispersant (OD), while the enhanced solubility of the low molecular weight PAHs by the OSD decreased the half-life of the alkylated PAHs in the OD. After 77 days of exposure at the sea surface, both the oils with

and without the OSD exhibited moderate weathering. Most of the source diagnostic indices maintained their source information, and the weathering indices indicated that evaporation, dissolution, and dispersion were the major weathering processes. The mass balance of the weathered oil was calculated using laboratory and mesocosm data, and the results demonstrate the importance of using a mesocosm for the production of environmentally realistic data.

**MP191 Metabolic response trajectories in the marine copepod *Calanus finmarchicus* exposed to chemically and mechanically dispersed oil** T.R. Storseth, SINTEF Fisheries and Aquaculture AS / Marine Resources Technology, SINTEF – Materials & Chemistry / Marine Environmental Technology; K.F. Degnes, SINTEF / Materials and Chemistry, SINTEF – Materials & Chemistry / Biotechnology; K. Tollefsen, Norwegian Institute for Water Research; A.J. Olsen, Norwegian University of Science and Technology / Department of Biology; T. Nordtug, B. Hansen, SINTEF Materials & Chemistry / Marine Environmental Technology. The marine copepod *Calanus finmarchicus* has an annual production of 300 million tons per year and is a vital part of the food chain the north Atlantic. Changes in the levels of this copepod could seriously affect several important commercial fish species, and as such it is a relevant model species for assessing effects of pollutants, such as those associated with increased oil exploration. Using NMR and MS based metabolomics approaches together with transcriptomics, we have studied the response to dispersed oil exposure in *C. finmarchicus* after a 96 hour exposure period, and subsequently a 96 hour recovery period. A comparison was made between mechanically and chemically dispersed oil at three different concentrations. Exposed samples were compared to a control group sampled simultaneously as a baseline for developing metabolism throughout the experiment. To evaluate effects of exposure principal component analysis (PCA) was used to check for differences. Polar extracts of low molecular weight metabolites were analyzed on a Bruker DRU 600 spectrometer, and the metabolic fingerprints were subjected to multivariate analysis in Matlab. Here PCA scores showed clear effects of exposure to high and medium concentrations of both types of oil. The effects were found to be separated into reversible and irreversible effects separated into PC1 and PC2, respectively. The separation of the reversible and irreversible effects into two separate PCs allowed for each of these to be described by their own one dimensional loading profiles. The loading profiles revealed levels of osmolyte/methyl donors betaine and homarine to be affected accompanied by a general perturbation of amino acids. MS data from the metabolome and lipidome of the same samples, and transcriptomics data will be presented together with assigned changes in the <sup>1</sup>H-NMR metabolic profiles.

**MP192 Multi-media monitoring of oil contamination – Four years after the Hebei Spirit Oil Spill** M. Kim, Korea Ocean Research and Development Institute / Oil & POPs Research Group; U. Yim, Korea Ocean Research and Development Institute / Oil & POPs Research Group, Korea Ocean Research & Development Institute; S. Ha, J. Jung, J. An, W. Shim, Korea Ocean Research and Development Institute / Oil & POPs Research Group.

**MP193 Assessing the risk of petroleum contaminants in migratory shorebirds** C.R. Labarrere, University of Saskatchewan / Toxicology Centre; C. Morrissey, University of Saskatchewan / Biology. Evidence suggests that an increased number of oil spills are leading to widespread exposure of shorebird populations to petroleum derivatives across their migratory range. We hypothesize that sublethal exposure to oil contaminants, specifically Polycyclic Aromatic Hydrocarbons (PAH) may be contributing to the decline of shorebird populations. This study is focused on understanding the extent to which shorebirds are exposed to PAHs from petroleum sources across their range in North and South America and their sensitivity to a range of PAH concentrations. The preliminary risk assessment involves evaluation of levels of PAH compounds with 2–5 rings (including USEPA's priority 16) in the environment (prey and sediment) from key stopover and wintering sites in Canada, United States, and Brazil to monitor the exposure and regional sources of contamination. The second phase of the study involves evaluating *in vitro* induction of ethoxyresorufin O-deethylase (EROD) activity in primary hepatocyte cell cultures of a target shorebird species, Sanderlings (*Calidris canutus*), to identify the toxic potency of PAH mixtures and sensitivity of this species to a wide range of doses. The risk characterization will be done by comparing levels in sediment and prey with reference LOAEL standard values and results from the *in vitro* study

to determine if PAHs are a potential concern to shorebird populations. The results from this research will guide future actions with regard to the conservation and sustainability of migratory shorebird populations in North and South America.

**MP194 Avian two-generation toxicity test method development using endosulfan as an estrogenic endocrine disrupting chemical in the Japanese quail** M. Quinn, US Army Institute for Public Health / Health Effects Research Program, US Army Public Health Command / Health Effects Research Program; C. McFarland, U.S. Army Public Health Command (provisional) / and Preventive Medicine, U.S. Army Public Health Command / Health Effects Research Program, U.S. Army Public Health Command, U.S. Army Center for HPPM; M.S. Johnson, US Army, Public Health Command / Health Effects Research Program, US Army Institute of Public Health / Health Effects Research Program, Army Institute of Public Health / Health Effects Research Program, US Army Ctr for Health Promotion & Preventive Med. / Health Effects Research Program; P. Browne, L. Touart, U.S. Environmental Protection Agency. We investigated the effects of oral and embryonic exposure to endosulfan in three successive generations of Japanese quail (*Coturnix japonica*) to help assess developing methodologies for an avian two-generation toxicity test. Endosulfan is an organochlorine insecticide used on crops worldwide, mainly on cotton, coffee, and tea. Its reproductive and developmental effects are well documented across taxa, and its effects as an endocrine disrupting chemical (EDC) are well understood in avian species. In this phase of method development, endosulfan was used as an estrogenic model EDC. The F0 generation of quail received endosulfan in their feed only; the F1 generation was exposed to endosulfan through maternal deposition as embryos and through feed from day of hatch throughout adulthood; the F2 generation was only exposed to endosulfan *in ovo*. Onset to reproductive maturity was measured as age of first egg laid in females and cloacal foam gland development in males. Copulatory behavior, egg production, fertility, eggshell strength and thickness, and sperm concentration and motility were assessed in adults from all generations, and growth was measured in F1 and F2 chicks. Data from this study will help to evaluate the U.S. Environmental Protection Agency's proposed two generation avian assay protocol for the Tier 2 Endocrine Disrupting Screening Program.

**MP195 Biochemical effects of dichlorvos on poultry birds** E.U. Ezeji, Department of Biotechnology, Federal University of Technology; I.N. Onwurrah, University of Nigeria, Nsukka / Department of Biochemistry. Dichlorvos is widely used as an insecticide to control household pests, in public health, protecting stored product from insects and control of parasites in livestock. Continuous use of dichlorvos and other pesticides in agriculture and domestic purposes may have adverse effects on humans and non-target animals. This study examines the biochemical effects of dichlorvos on poultry birds. Results of this study show significant reductions ( $P < 0.05$ ) in liver cytoplasmic and membrane bound cholesterol, triglycerides and total lipids in the birds fed on pesticide contaminated diet, as well as reduction in levels of glutathione and glutathione S-transferase activity. There was also a significant increase in lipid peroxidation in the birds exposed to pesticide. Results of this test indicate an induction of oxidative stress on poultry birds exposed to dichlorvos.

**MP196 Bone Lead Concentrations in American Woodcock from Seven U.S. States** J.C. Franson, US Geological Survey / National Wildlife Health Center. Lead concentrations were determined in wing bones of more than 1,500 American woodcock (*Scolopax minor*) from Minnesota, Wisconsin, Michigan, New York, Vermont, New Hampshire, and Maine. Bone lead concentrations in woodcock differed by age, sex, and location. Lead concentrations were significantly greater in adults than juveniles, and adult females had greater lead concentrations than adult males. Among the states, lead concentrations in wing bones of both adult and juvenile woodcock were highest in Maine, New Hampshire, and Vermont, intermediate in New York, and lowest in Michigan, Wisconsin, and Minnesota. The lead levels in bones of woodcock in this study and others were higher than those generally reported in a variety of other bird species, perhaps because of lead contained in soil and earthworms ingested by woodcock as they feed. However, although the ingestion of spent lead shot has not been reported in woodcock, at least one report has suggested that the pattern of stable lead isotope ratios in bones of woodcock with elevated lead concentrations is consistent with exposure from ingested shot. Woodcock populations in both the eastern



and central management units in the United States have exhibited long-term declines since 1960s. Particularly because of recent interest and concern about exposure of upland game birds to spent lead shot, further studies are needed to determine the contribution, if any, of ingested lead shot to elevated bone lead concentrations in American woodcock.

**MP197 Cadmium Transport and Accumulation in Xylem of Two Cultivars of Soybean Differing in Tissue Accumulation and Distribution** T. Jennett, University of Guelph / school of environmental science; B. Hale, University of Guelph / School of Environmental Sciences. Accumulation of Cd in soybean seed is a heritable trait, the expression of which is modified by bioavailability of soil Cd and water use by the plant. Cadmium is xylem-mobile – the main transport pathway for Cd from source to sink tissues. OAC Bayfield and OAC Champion (lower and higher Cd accumulating soybean cultivars, respectively) were grown in Cd-amended hydroponic nutrient solution and the [Cd] transported in xylem fluid and accumulated in tissues was measured at three life stages (flowering, pod initiation, and seed fill). OAC Champion retained 94% (vs. 64% for OAC Bayfield) of total plant-accumulated Cd in the root tissues, while the [Cd] in its xylem sap was eleven-fold less concentrated than OAC Bayfield by seed fill life stage. Though the movement of Cd to shoots was more restricted in OAC Champion, the [Cd] in some seed tissue still approximated or exceeded 0.1 mg Cd · kg<sup>-1</sup>, suggesting that internal physiological processes exert considerable control over Cd concentration in beans, rather than the concentration of Cd that is accumulated by the roots, presumably much of which is apoplastic and thus non-specific. Intervarietal differences in water use efficiency may be the driver of the internal control.

**MP198 California's Catastrophic Wildland Fires: Increasing the Risks of Burning Hotter, Faster, and Higher** C. Menzie, Exponent, Inc., Exponent / EcoSciences; T.L. Deardorff, Exponent / Senior Managing Scientist; J. Ma, Exponent; M. Edwards, Exponent / Environmental and Human Health Consulting. Decades of aggressive fire suppression by the U.S. Forest Service (USFS) have drastically changed the look and fire behavior of Western forests and rangelands. Our forests have increased vegetative densities and possess less fire-resistant tree species. As a result, today's wildfires typically burn hotter, faster, and higher into the forest canopy than fires of the past. In turn, the number of catastrophic wildfires has increased dramatically over the past few decades. We recently examined environmental conditions surrounding two California wildfires. One – the 2007 Grass Valley Fire – occurred in the San Bernardino National Forest (SBNF) located in a relatively dryer region of southern California; the other – the Sims Fire – occurred in the Six Rivers / Shasta Trinity National Forest (SR/STNF) located in a relatively wetter region of the state. The total acreage of burned forest has increased since the 1970s for the SBNF and since the 1980s for the SR/STNF. Acreage of burned forest in both regions peaked in the 2000 – 2010 decade. Concomitantly, there was an increase in the size of fires in the two regions during this past decade. The pattern of increased acreage burned for both dry and wet regions of California led us to explore the risk factors contributing to the occurrence of catastrophic wildfires in these two California national forests. We examined three risk factors as they relate to the occurrence of catastrophic wildfires: frequency of initiating events (e.g., the spark), dryness as indicated by rainfall or periods of drought, and the condition of the forest including fuel accumulation. We considered each of the possible risk factors as a contributing factor by examining temporal trends in those factors in comparison to the trends in acreage burned. Our analysis indicates that while a myriad of factors contribute to the likelihood of catastrophic wildfires in California forests, of salient importance is the moisture content of the forest, the condition of the pre-fire forest, and fuel accumulation. The latter finding appears to be related to the historical forest management practice of fire suppression. This finding is consistent with assessments made by the USFS and the current emphasis on fuels management and a desire to return forests to more natural fire regimes.

**MP199 Contaminant Exposure, Food Web Transfer and Potential Effects to Ospreys (*Pandion haliaetus*) Nesting in Chesapeake Bay Regions of Concern** R.S. Lazarus, USGS Patuxent Wildlife Research Center / USGS, USGS-Patuxent Wildlife Research Center / Beltsville Laboratory; B.A. Rattner, USGS-Patuxent Wildlife Research Ctr / USGS, USGS-Patuxent Wildlife Research Center / Beltsville Laboratory, USGS-Patuxent Wildlife Research Ctr / Beltsville Laboratory; P.C. McGowan, U.S Fish and Wildlife Service Chesapeake Bay Field Office; R.C. Hale, Virginia Institute

of Marine Science / Environmental & Aquatic Animal Health, Virginia Institute of Marine Science / Environmental Science Department; M. Ottinger, University of Maryland / Department of Animal and Avian Sciences. In 2000-2001, the last large-scale Chesapeake Bay ecotoxicological study of ospreys was undertaken in regions of concern (Baltimore Harbor/Patapsco River, Anacostia/middle Potomac Rivers, Elizabeth River). A decade later, osprey egg samples were collected from the Anacostia/middle Potomac Rivers (*n*=13), Baltimore Harbor/Patapsco River (*n*=7), and Poplar Island reference site (*n*=4) to monitor spatial and temporal trends in the concentrations of organochlorine pesticides and metabolites, PCB congeners, polybrominated diphenyl ethers (PBDEs), and other brominated flame retardants. Concentrations of *p,p*-DDE and other organochlorine pesticides in osprey eggs have declined from values reported in the 1970's, while PCBs remain elevated. For example, the geometric mean of total PCBs in eggs collected from the Patapsco in 2000 was 7.25 µg/g wet weight, and in 2011 was 7.77 µg/g, with hotspots in Curtis Creek ranging up to 34.9 µg/g. On the Anacostia/middle Potomac, PCB concentrations seemingly declined between 2000 and 2011 (9.28 vs. 4.84 µg/g), but were greater than observed on Poplar (1.55 µg/g). Over the past decade, levels of PBDEs have decreased in both Baltimore Harbor (320 to 184 ng/g) and the Anacostia/middle Potomac (725 to 344 ng/g), but are notably greater compared to Poplar (61.9 ng/g). Alpha-hexabromocyclododecane (α-HBCD) was present in every sample from regions of concern and Poplar alike (0.36 to 3.03 ng/g). Residues of PBDEs and α-HBCD peaked in samples collected in the vicinity of the Blue Plains wastewater treatment plant on the Potomac and decreased moving 55 km downriver. The brominated flame retardants (e.g., bis (2,4,6-tribromophenoxy)ethane; 2-ethyl 2,3,4,5-tetrabromobenzoate; tetrabromophthalate) were detected in 2000 and 2011 samples from Baltimore Harbor/Patapsco River and the Anacostia/Potomac River. Low levels of the antiseptic metabolite methoxy triclosan were present in eggs from the Anacostia/Potomac (1.15 to 6.29 ng/g). In 2011, osprey productivity was adequate to maintain stable populations (>1.15 fledglings/active nest) at all study sites. Pesticides, PCBs and flame-retardants are being analyzed in the principal fish species consumed by ospreys to examine biomagnification in the food web. A suite of pharmaceuticals are being quantified in water, and fish and osprey plasma to examine exposure and potential hazard.

**MP200 Effects of 1,2,5,6,9,10-HBCD dietary exposure on reproductive measures in Japanese quail** P.F. Henry, U.S. Geological Survey / Patuxent Wildlife Research Center; N.K. Karouna-Renier, Y. Chen, D. Sprague, J. Green, C. Maddox, USGS-Patuxent Wildlife Research Center. The organohalogen, 1,2,5,6,9,10-hexabromocyclododecane (HBCD) is embedded as an effective brominated flame retardant into polystyrene insulating foams for construction, upholstery, and electronics devices. Recent concerns are mounting surrounding the ecological distribution and accumulation of HBCD in wildlife as well as in human tissue. HBCD bioaccumulates and biomagnifies, with isomeric concentrations detected globally and distributed along aquatic and terrestrial avian food chains. As an endocrine active compound, HBCD has the potential to bind to androgen, estrogen, progesterone, and thyroid receptors, and to act as a competitive inhibitor. Effects on Japanese quail (*Coturnix japonica*, JQ) of dietary exposure to 5 environmentally relevant doses of HBCD were investigated over 2 generations. Onset to egg laying, fertility, behavior, overall productivity measures, and hormone concentrations are reported for first generation quail initially exposed to HBCD at the onset of reproduction and for second generation quail exposed during embryonic development through sexual maturation.

**MP201 Effects of early exposure to BDE-99 on growth, thyroid hormones, and photo-induced reproductive development in European starlings** M.L. Eng, T.D. Williams, Simon Fraser University; R.J. Letcher, Carleton University / Department of Chemistry, Environment Canada / Science and Technology Branch, Ecotoxicology and Wildlife Health Division, Ecotoxicology and Wildlife Health Division, Science and Technology Branch / Environment Canada; J. Elliott, Environment Canada / Pacific Wildlife Research Centre, Science and Technology Branch, Environment Canada / Pacific Wildlife Research Centre, Environment Canada / Wildlife Toxicology, Environment Canada / Canadian Wildlife Service. 2,2',4,4',5-Pentabromodiphenyl ether (BDE-99) is a brominated flame retardant congener that has pervaded global food chains, being reported in avian egg and tissue samples throughout the world. In birds, early life stages are the most sensitive to environmental conditions, and exposure to contaminants during the nesting period may have critical effects at concentrations



much lower than those required to affect adults. These effects of exposure may not be evident until reproductive maturity, necessitating long term studies to assess fitness implications. The objectives of this study were to examine the long-term effects of early exposure to BDE99 in a model passerine species, the European starling (*Sturnus vulgaris*). We orally dosed free-living nestlings for the duration of the nesting cycle to environmentally relevant, sublethal levels of BDE99 (0-173.8 ng/g bw/day). To monitor long-term effects of exposure, birds were brought into captivity just prior to fledging and raised to reproductive maturity using photoperiod manipulations. To assess reproductive development we recorded moult score and bill colour, and conducted laparotomies in males to measure testes size. We took blood samples over the course of development to measure thyroid hormones and sex steroid hormones. At our dose levels, there was some evidence that early exposure to BDE-99 resulted in delayed reproductive development in male European starlings, however these effects were not significant. There were no other effects on growth or physiology.

**MP202 Effects of Pesticide Applications in the Klamath Basin on the Health and White-nose Susceptibility of Foraging Bats** T. Adams, U.S. Fish and Wildlife Service; C. Johnson, U.S. Fish and Wildlife Service / Environmental Contaminants Division. Bats fill a vital role in the agricultural ecosystem. It has been determined that a single colony of 150 bats will consume 1.3 million pest insects a year with a lactating females consuming two thirds their body mass each night. Consequently, bats likely save the agricultural industry 3 billion dollars annually. This characteristic, however, places them at risk for pesticide exposure either by ingesting contaminated prey, drinking contaminated water or direct application. This study evaluates the health and pesticide load of the largest colony of roosting Brazilian free-tailed bats in the Pacific Northwest prior to its exposure to white-nosed syndrome (WNS). WNS is a disease associated with 5.7 to 6.7 million deaths in North American bats and is distinctive by its fungal growth on the bats nose and wings. Currently, it is uncertain if pesticides play an indirect role in the spread of WNS and subsequent population loss. It is a unique opportunity to study the before and after effects of WNS on bats before its inevitable arrival to California and Oregon. This data can be used to determine what biological parameters make roosting bats more susceptible to WNS. We collected Brazilian free-tailed guano from three sites in the Klamath Basin for pyrethroid insecticide analysis. Two pyrethroids were detected. Bifenthrin was detected at all three sites at 0.97, 1.46 and 1.08 ng/g. Permethrin was detected at site two and three at 2.50 and 7.76 ng/g.

**MP203 Effects of pesticides on cell migration in human breast cancer cells** A. Jesionowski, J. Schultz-Norton, Millikin University. Environmental chemicals have been suspected to have roles in the metastasis of reproductive cancers. Six of these compounds which are used as pesticides for agricultural purposes were selected for this experiment. Estrogen receptor-positive (MCF-7 cells) and estrogen receptor-negative (MDA-MB-231 cells) human breast cancer cells were plated, a scratch wound was created amongst them, and the migration of the cells across the wound was measured in 24-hour increments after treatment with the pesticides. ImageJ software was used to analyze gap distances and determine percent wound recovery, and statistical significance was determined using SPSS. Interestingly, although there are similarities in the structures of the three organochlorine pesticides analyzed, strong differences in their abilities to affect cell migration were observed. These in vitro studies indicate that exposure of cancerous cells to pesticides may alter their growth, thus having implications for farming communities.

**MP204 Estradiol-responsive gene expression does not reflect circulating steroid levels in Japanese quail (*Coturnix japonica*) exposed to 17 $\beta$ -trenbolone** Y. Chen, Virginia Tech / Department of Fisheries and Wildlife Sciences, USGS Patuxent Wildlife Research Center, USGS Patuxent Wildlife Research Center / Patuxent Wildlife Research Center, U. S. Geological Survey / Patuxent Wildlife Research Center; N. Karouna-Renier, USGS Patuxent Wildlife Research Center / Beltsville Lab / U.S.G.S.-Patuxent Wildlife Res. Ctr / Beltsville Lab; P.F. Henry, U.S. Geological Survey / Patuxent Wildlife Research Center; C. Maddox, Patuxent Wildlife Research Center, USGS / Beltsville Lab; D. Sprague, USGS Patuxent Wildlife Research Center. The expression of sex-steroid responsive genes is commonly used as an indicator of endocrine disruption by estrogenic chemicals. However, studies of their utility for monitoring the effects of androgenic chemicals are limited, particularly in birds. We therefore evaluated the efficacy of several hepatic estradiol-responsive genes [vitellogenin II (VTG),

very low-density apolipoprotein II (ApoVLDLII), and avian perivitelline membrane glycoprotein (ZP1)] as indicators of endocrine alterations in Japanese quail and compared their expression to levels of circulating steroid hormones. Quail were exposed through diet to the synthetic anabolic steroid 17 $\beta$ -trenbolone over three consecutive generations. F0 pairs were exposed to treated diet from 6 weeks, F1 were exposed *in ovo* and to treated diet from hatch, and F2 were exposed *in ovo* only. Circulating testosterone levels decreased dose-dependently in F0 females and were significantly lower in F0 and F1 females received feed containing 20 and 40 ppm trenbolone. In females, circulating estradiol levels were not significantly affected by trenbolone exposure although VTG mRNA levels were significantly suppressed in the F0 generation. In contrast, circulating testosterone levels remained similar across all males, but estradiol levels were significantly elevated in trenbolone exposed F0 and F1 males. However, the hepatic mRNA levels of VTG in these males were not significantly different from the controls. Likewise, the expression patterns of other estradiol-responsive genes (ApoVLDLII, ZP1) in male and female birds did not reflect hormone levels. Previous studies in fish have hypothesized that androgen agonists can trigger a negative feedback response in females that results in reduced testosterone synthesis and in turn limits the synthesis of estradiol, thereby affecting expression of estradiol-responsive genes. However, our data suggest that a different mechanism is responsible for regulating these genes in Japanese quail since expression did not reflect estradiol levels in either females or males.

**MP205 Evaluation of an avian two-generation toxicity test using trenbolone as an androgenic endocrine disrupting chemical in Japanese quail** M. Quinn, US Army Institute for Public Health / Health Effects Research Program, US Army Public Health Command / Health Effects Research Program; C. McFarland, U.S. Army Public Health Command (provisional) / and Preventive Medicine, U.S. Army Public Health Command / Health Effects Research Program, U.S. Army Public Health Command, U.S. Army Center for HPPM; A. Jackovitz, U.S. Army Public Health Command; M.S. Johnson, US Army, Public Health Command / Health Effects Research Program, US Army Institute of Public Health / Health Effects Research Program, Army Institute of Public Health / Health Effects Research Program, US Army Ctr for Health Promotion & Preventive Med. / Health Effects Research Program; P. Browne, L. Touart, U.S. Environmental Protection Agency. The U.S. Environmental Protection Agency is implementing an Endocrine Disruptor Screening Program (EDSP) comprised of a battery of Tier 1 screening assays and Tier 2 tests. One of the Tier 2 tests under development is a two-generation avian assay using Japanese quail (*Coturnix japonica*). This assay serves to evaluate potential population effects of endocrine modulating compounds on avian species, and represents terrestrial, oviparous wildlife species in general. This study used trenbolone as a model androgenic endocrine disrupting chemical to evaluate the proposed test method further for its ability to detect adverse effects, characterize dose responses in a two-generation paradigm, and to develop and optimize a protocol for the avian two-generation assay for characterizing endocrine disruptors. Trenbolone acetate is a growth promoter that had been widely used for beef production. The toxicity of its androgen active metabolite 17 $\beta$ -trenbolone has been well characterized in Japanese quail. The F0 generation was exposed to trenbolone acetate in feed only, the F1 generation was exposed *in ovo* and via feed, and the F2 generation was only embryonically exposed. Onset to reproductive maturity was measured as age of first egg laid in females and cloacal foam gland development in males. Copulatory behavior, measures of fecundity, circulating steroid hormones, eggshell strength and thickness, and sperm concentration and motility were assessed in adults from all generations. Growth was measured in chicks from the F1 and F2 generations. This work will help to determine the value added by examining endocrine endpoints over two generations in comparison to tests that only include one generation, such as the Ecological Effects Test Guidelines OPPTS 850.2300 Avian Reproduction Test.

**MP207 Examining Zn redistribution in the gut of *L. terrestris*** K.E. Linford, Towson University / Urban Environmental Biogeochemistry Laboratory; R. Casey, D.R. Ownby, Towson University / Urban Environmental Biogeochemistry Laboratory, Towson University / Department of Chemistry; S.M. Lev, Towson University / Urban Environmental Biogeochemistry Laboratory, Towson University / Professor. Enriching *L. terrestris* with  $^{68}\text{Zn}$  can enable us to observe the subsequent decrease of  $^{68}\text{Zn}$ :  $^{66}\text{Zn}$  in *L. terrestris* organ tissues, which previous work has shown is related to soil zinc

bioavailability. Zn spiked food was studied as a source of enrichment. Earthworm tissue  $^{68}\text{Zn}$ : $^{66}\text{Zn}$  ratios were not enriched after feeding the worms a  $^{68}\text{Zn}$ -enriched diet. We hypothesized that the lack of enrichment was due to redistribution of  $^{68}\text{Zn}$  between diet and soil in the gut and that the competition of soil exchange sites for Zn resulted in the  $^{68}\text{Zn}$  being less available to gut metal receptors. *L. terrestris* consume both soil and organic matter. When the  $^{68}\text{Zn}$ -spiked food is consumed, a period of redistribution, within the gut, of both  $^{68}\text{Zn}$  and background Zn may occur. Zn that is dissolved in the gut solution is likely the more available fraction and available to interact with metal uptake receptors in the gut while Zn bound to soil in the earthworm gut is likely less available. We hypothesize that if increasing amounts of soil were added to the gut system that increasing amounts of  $^{68}\text{Zn}$  would exchange with cations on the soil surface. The  $^{68}\text{Zn}$  will largely be bound to soil and unavailable to interact with uptake proteins in the gut. As more  $^{68}\text{Zn}$  is sequestered by the soil, the  $^{68}\text{Zn}$ : $^{66}\text{Zn}$  ratio will decrease and less  $^{68}\text{Zn}$  may be available to the organism. To examine the fate of  $^{68}\text{Zn}$  in the worm's gut, a two-part simulated earthworm gut (SEG) experiment was conducted. The SEG is an enzyme solution composed of major enzymes found in the worm gut. In the first part of the experiment, increasing amounts of soil were digested in SEG fluid along with a constant amount of  $^{68}\text{Zn}$ -spiked food. The ratio of  $^{68}\text{Zn}$ : $^{66}\text{Zn}$  analyzed in the SEG solution decreased with increasing soil content. The total Zn levels in solution decreased with increasing soil content.  $^{68}\text{Zn}$  in the food item may be rendered less available to gut metal receptors due to sequestration by soil ligands in the gut. Next, a kinetic experiment was run to determine the time over which the Zn sequestration occurs. A  $^{68}\text{Zn}$ -enriched oat bran and artificial soil were digested with SEG solution and removed for metals analysis at times ranging from 1-24 hours. The  $^{68}\text{Zn}$ : $^{66}\text{Zn}$  ratio declined within the first eight hours and then remained constant. This suggests that Zn redistribution in the gut of *L. terrestris* occurs in the first several hours of gut residence time.

#### MP208 Exposure and effect evaluation of amphibole asbestos in small mammals

S. Robinson, Golder Associates / Toxicology & Risk Assessment; J.M. Clark, Parametrix, Golder Associates, Inc. / Environmental Sciences; J. Volosin, Anchor QEA; A. Fairbrother, Exponent / EcoSciences, Exponent, Inc. / EcoSciences; C. Claytor, GEI Consultants, Inc.; M. Garner, Northwest ZooPath; J. Burris, CDM Smith. Sampling of Libby Amphibole (LA) asbestos in environmental media, including soil, water, and tissue, has been occurring at the Libby Asbestos Superfund Site, Operable Unit 3 (OU3), in Libby, Montana to support the preparation of an ecological risk assessment as part of a Remedial Investigation and Feasibility Study. The larger site includes the property in and around a former open pit vermiculite mine that contains LA. There are no published toxicity reference values or benchmarks established for asbestos in the scientific literature that can be used to evaluate potential risk to terrestrial ecological receptors at the Site. A study was designed to evaluate LA exposure in burrowing small mammals and determine the potential for adverse effects from areas with the highest measured asbestos concentrations in forest duff. The small mammal species targeted for tissue collection, the deer mouse (*Peromyscus maniculatus*) and the southern red-backed vole (*Clethrionomys gapperi*), were selected because both have a small home range, burrow and forage on the ground and have small bodyweights, thus ensuring maximal exposure to soil and forest duff containing LA. The potential for adverse effects from asbestos was determined through comparison of the results from external exams, necropsy and histological examination of target tissues for Site and reference animals. Target tissues for histology were determined from a review of existing rat and mouse toxicity studies conducted with amphibole and chrysotile asbestos to support human health toxicity assessment, and included; (i) complete pulmonary tract; (ii) complete gastrointestinal tract; (iii) thyroid; and (iv) adrenal glands. Baited live traps were used to collect target species at the Mine Site and in the upwind reference collection areas between 27 August and 2 September 2009. Red backed-voles were not captured in any of the trapping areas. Animal exams, necropsies and tissue harvesting for histology were conducted in an offsite laboratory. No evidence of abnormalities or pathology attributable to asbestos was identified from the animal external examinations, necropsies or target tissue histological evaluations. We concluded that though the deer mice were exposed to high LA concentrations, there were no discernible effects to the animals that would impair their general health status, growth, survival or reproduction.

#### MP209 Health and Reproductive Impairments in Colonial Waterbirds in the Saginaw Bay and River Raisin Areas of Concern

K. Grasman, Calvin College / Department of Biology, Calvin College / Department of Biological; S. Fuhrman, M. Langeland, M. Mc Rae, Calvin College / Department of Biology; L. Williams, US Fish and Wildlife Service. This assessment investigated effects of contaminants on reproduction, growth, and immunological health of fish-eating birds in the Saginaw Bay and Raisin River Areas of Concern (AOCs) as part of the Great Lakes Restoration Initiative-Fish and Wildlife Service AOC program. In Saginaw Bay field studies were conducted during 2010-2012 at two herring gulls colonies (Confined Disposal Facility (CDF) in the southern bay and Little Charity Island in the outer bay), two Caspian tern colonies (CDF and Charity Reef) and one black-crowned night heron colony (CDF). At the River Raisin AOC, herring gulls were studied at the Detroit Edison Monroe Power Plant on the western shore of Lake Erie. Reference sites were located in the lower St. Mary's River (Pipe Island Twins for gulls and Two Tree Island for terns) and on Chantry Island, Lake Huron (herons). Gull nests were marked during egg-laying, and embryonic viability was assessed during mid/late egg incubation using an embryonic viability detector sensitive to heartbeat and movement. Embryonic nonviability rates in herring gulls in the Saginaw Bay and River Raisin AOCs (4.5-13%) were higher than at reference sites (< 2%). Infertile eggs and embryos that died at various stages of development were found at all sites. Total nonviability was most influenced by infertility on L. Charity and at Monroe. In 2012 a herring gull chick was found at Monroe with a crossed bill, a deformity associated with PCBs and dioxins in previous studies. Herring gull chicks in Saginaw Bay had marginally good survival, and Caspian tern chicks had poor to marginal survival, a concern for this state-threatened species. Herring gulls at Monroe experienced poor chick survival in all 3 years, with complete reproductive failure during 2010. Mean phytohemagglutinin skin responses for T-cell mediated immunity were suppressed dramatically compared to reference sites in young herring gulls (50-58%), Caspian terns (48-53%), and black-crowned night herons (33%) in the Saginaw Bay AOC. This immune response was suppressed 65% in herring gulls at the River Raisin AOC. Ongoing immunological, developmental, and reproductive impairments in fish-eating birds at these AOCs are consistent with previous studies on the effects of persistent pollutants such as PCBs in Great Lakes birds, although other stressors may be contributing to reproductive effects.

#### MP210 Histology as a biomarker of environmental pollution in 2 two frog species in the Kruger National Park, South Africa

G. Wagenaar, University of Johannesburg / Department of Zoology; C. van Dyk, University of Johannesburg / Department of Zoology, Centre for Aquatic Research; W. Vlok, BioAssets. The aim of the research was to use histology to evaluate the health status of 2 frog species in the Kruger National Park, South Africa. Histology showed to be a suitable biomarker for environmental pollution. Ten specimens were collected of the two frog species *Tomopterna cryptotis* and *Breviceps adspersus* respectively from two pans (Skukuza and Tsokoane). Selected organs of both species were processed for light microscopy analysis using standard techniques for Haematoxylin and Eosin staining. The light microscopy analysis showed that the general tissue architecture of the different target organs of both species were intact and in a functional state. Specific histological alterations were identified in the liver and kidney samples of both species, from both sampling sites. Some of the specimens from both sites also showed focal areas of hepatocyte degeneration and necrosis. In other areas, necrotic cells were characterized by dark eosinophilic cytoplasm and pyknotic nuclei. Only a few histological alterations were identified in the kidneys of *T. cryptotis*. These included hydropic change of the tubular epithelial cells of two specimens, an increased presence of inter-tubular blood in one specimen from Skukuza and the presence of melano-macrophage centres in one specimen from Tsokoane. The liver samples in *B. adspersus* showed the characteristic hepatic cord organization and the general hepatic architecture was visible in all liver samples. Some of the specimens from both sites also showed focal areas of liver degeneration and necrosis. These areas were mainly in peri-vascular tissue regions and were characterized by pale eosinophilic hepatocytes and nuclei that did not take up Haematoxylin. In other areas, necrotic cells were characterized by dark eosinophilic cytoplasm and pyknotic nuclei. A few histological alterations were also identified in the kidneys of *B. adspersus*. Nuclear pleomorphism was observed in one of the specimens from Skukuza. A mild inflammatory response, characterized by the infiltration of mono-nuclear leukocytes was identified in one specimen from Tsokoane. No histological alterations were

identified in any of the stomach, lung, heart or skin samples analysed. The results obtained in this study suggest that histology could be a useful bio-indicator for pollution.

#### **MP211 Historic Lead Contamination on Southeast Farallon Island:**

**Impacts to Nesting Seabirds** K. Aceituno, U.S. Fish and Wildlife Service / Fish and Wildlife Biologist, U.S. Fish and Wildlife Service / Environmental Contaminants Division; C. Johnson, U.S. Fish and Wildlife Service; G. McChesney, U.S. Fish and Wildlife Service / Farallon National Wildlife Refuge; R. Bradley, P. Warzybok, PRBO Conservation Science. The Farallon Islands are located 28 miles west of San Francisco, and support the largest breeding population of marine birds in the contiguous United States. However, many species have been experiencing declines in reproductive performance over the past several decades. We investigated historic lead contamination on Southeast Farallon Island and the impact it may be having on nesting seabird populations. We sampled breast feathers from Cassin's auklet (*Ptychoramphus aleuticus*) and western gull (*Larus occidentalis*) chicks from areas on the Island with and without known lead contamination in the soil. Additionally, a corresponding soil sample was taken from each Cassin's auklet nest box. Lead concentrations in feathers from both species taken from contaminated areas are significantly higher than those from non-contaminated areas. Moreover, lead concentrations in some samples approached levels found on the Midway Atoll, an Island with a well documented history of lead contamination.

#### **MP212 In ovo exposure to 1-nitropyrene: Developmental, biochemical and behavioral changes in white leghorn chicken hatchlings**

M. Bradley, L. Huang, J. Rutkiewicz, K. Mittal, J. Head, S. Chernyak, S. Batterman, University of Michigan; N. Basu, University of Michigan / Department of Environmental Health Sciences, University of Michigan / Department of Environmental Health. Polycyclic aromatic hydrocarbons (PAHs) are well-studied combustion products that are ubiquitous, bioaccumulative, mutagenic and carcinogenic. Nitro-substituted PAHs (nitro-PAHs) are also common combustion products, but much less is known about their fate and toxicity than unsubstituted PAHs. The objective of this study was to focus on 1-nitropyrene and characterize its tissue distribution as well as effects at the biochemical (neurochemical, oxidative stress), behavioral, and developmental levels in white leghorn chicken hatchlings following in ovo injections. Eggs were injected with 1-nitropyrene (0, 0.001, 0.01, 0.03, 0.1, 0.3, 1, 3, 10, 20 mg/mL peanut/soybean/sesame oil) at incubation day 3 and incubated until chicks hatched at day 21. While there was embryonic mortality, this did not follow a clear dose-response pattern. We tested one day old and one week old chicks for righting response, startle response, balance on angled balance beams, and gait patterns, and results thus far indicate no associations related to N-PAH exposure. Next steps will include measuring a series of neurochemical biomarkers (NMDA and GABA receptor levels; glutamine synthetase and glutamic acid decarboxylase enzyme activity) in the brain, and oxidative stress markers (GST and catalase activity) in brain, liver, and kidney. In terms of tissue distribution, a small group of eggs was injected with a mixture of 6 nitro-PAH congeners (low dose mixture and high dose mixture). In incubation day 18 embryos, most total nitro-PAH were found in brain (Low: 3.45 µg/g tissue; High: 29.56 µg/g tissue), liver (Low: 3.97 µg/g tissue; High: 30.58 µg/g tissue), and kidney (Low: 11.05 µg/g tissue; High: 5.57 µg/g tissue), though metabolism could not be assessed due to difficulties distinguishing between injected congeners and their metabolites.

#### **MP213 Incorporating Bioaccessibility in Estimating Metal Toxicity from Incidentally Ingested Soil**

T. Schlekert, ARCADIS; J. Meyer, Arcadis U.S., Inc., ARCADIS; C. Meyer, D. Jones, ARCADIS. Dietary exposure models are often used as the primary line of evidence in the assessment of risk to wildlife under the ecological risk assessment framework. Dietary exposure models use measured or modeled prey tissue concentrations and measured sediment (or soil) concentrations to evaluate exposure through the dietary pathway and incidental sediment ingestion. For wildlife with high incidental sediment ingestion rates, incidental sediment ingestion typically contributes a large portion of the dose of metals that do not bioaccumulate. In that situation, assessing the incidental sediment ingestion pathway using total metal concentrations without consideration of bioaccessibility may lead to an overestimation of risk. This was the case at the Tennessee Valley Authority Ash Recovery Project in Kingston, Tennessee, where a screening assessment showed risk to wildlife for metals that are naturally-occurring components of

sediments (aluminum, copper, iron, lead, manganese, and vanadium). These metals are expected to be present in sediment in the following four forms/fractions that are likely bioaccessible to wildlife: water-soluble, exchangeable, acid soluble, and easily-reducible. These fractions were quantified for sediment at the site for other objectives using a sequential extraction procedure (SEP) method. Concentrations of those metals in the four bioaccessible fractions were used together with total concentrations in the samples to estimate sediment bioaccessibility fractions and refine the dose estimates. Bioaccessibility fractions in site sediments were calculated to be: 1.2, 2.0, 6.2, 4.7, 75, and 2.5 percent for aluminum, copper, iron, lead, manganese, and vanadium, respectively. Dose estimates calculated after adjusting for bioaccessibility more closely correlated to measured tissue concentrations than dose estimates calculated without considering bioaccessibility.

#### **MP214 Influence of Food Web Dynamics on Mercury Bioaccumulation in Nesting Seabirds**

K. Aceituno, U.S. Fish and Wildlife Service / Fish and Wildlife Biologist, U.S. Fish and Wildlife Service / Environmental Contaminants Division; C. Johnson, U.S. Fish and Wildlife Service / Environmental Contaminants Division; G. McChesney, U.S. Fish and Wildlife Service / Farallon National Wildlife Refuge; R. Bradley, PRBO Conservation Science. The Farallon Islands are located 28 miles west of San Francisco and support the largest colony of breeding seabirds in the contiguous United States; however, reproductive performance in many seabird species has been declining over the past several decades. Mercury remains one of the most pervasive contaminants along California's coast due to historic mining and atmospheric deposition. To investigate mercury bioaccumulation in seabirds we analyzed eggs from three species nesting on Southeast Farallon Island: Cassin's auklet (*Ptychoramphus aleuticus*), rhinoceros auklet (*Cerorhinca monocerata*) and pigeon guillemot (*Cepphus columba*). In addition to eggs, we analyzed a variety of fish and invertebrates that nesting seabirds feed on. To assess trophic position and feeding location egg and diet samples also underwent carbon and nitrogen stable isotope analysis. Results indicate both pigeon guillemot and rhinoceros auklet eggs have mercury concentrations that exceed the lowest observed adverse effects level of 0.50 µg/g (fresh wet weight) for impaired avian egg hatchability, suggesting that seabirds breeding on Southeast Farallon Island are being exposed to levels of mercury that could be contributing to observed population declines. Further, isotopic analysis indicates that mercury bioaccumulation in seabirds is impacted by both trophic position and feeding location.

#### **MP215 Insight into Persistent Organic Pollutant (POP) Fate in British Columbia Grizzly Bears as Revealed by Life History Factors**

J. Christensen, Stantec Consulting Ltd. / Toxicology; M. Yunker, Contractor; M. MacDuffee, Raincoast Conservation Society; P.S. Ross, Fisheries and Oceans Canada / Institute of Ocean Sciences. While life history traits are often viewed as confounding factors in the interpretation of Persistent Organic Pollutant (POP) levels, patterns and effects in wildlife, their application to models of POP exposure and fate has seldom been employed. Here, we document the lessons learned from our grizzly bear research, where life history traits were used as tools to understand the influence of trophic level, food web (terrestrial versus marine), global versus local sources, metabolic biotransformation capacity, and food digestibility on overall POP accumulation in grizzly bears. These tools included 1) seasonal reliance on salmon by coastal bears, 2) hibernation, and 3) diurnal changes, from vegetation to salmon, in the fall diet of coastal bears. The seasonal salmon reliance of some bears revealed a divergence from a terrestrially-based diet in spring characterized by polybrominated diphenyl ether (PBDE) exposures, to a marine-based salmon diet in the fall characterized by polychlorinated biphenyl (PCB) exposures. Biomagnification factors, calculated by comparing salmon-eating bears to salmon, were low (< 1), suggesting that even the most bioaccumulative POPs (e.g., PCB-153) were not magnifying in bears. Overall, the depuration of PCBs via biotransformation is important in explaining the loss of POPs in bears (explaining ~40% of loss), but excretion prior to hibernation appeared to be a more important factor (explaining ~60% of loss). By examining differences in POP patterns between pre- and post-hibernation bears, we determined that while diet (food web) dictates POP patterns during a feeding phase, metabolism drives patterns during a fasting phase. Hibernation therefore revealed the duality of POP-associated health risks to these hibernating bears, including a fasting-associated increase in some POP concentrations, and a prolonged accumulation of highly reactive POP metabolites, since grizzly bears do not excrete during



hibernation. Finally, diurnal dietary changes from salmon to vegetation by coastal grizzly bears revealed that salmon consumption does lead to elevated exposure to most POPs; however, the consumption of vegetation following a salmon meal results in a dramatic vegetation-associated drawdown of those marine-derived POPs. Collectively, this research highlights the importance of integrating ecology with toxicology at the study design stage in order to comprehensively evaluate POP-related exposure, fate and health risks.

**MP216 Mercury in Bats from the Northeastern United States** D. Yates, D.C. Evers, Biodiversity Research Institute; T.H. Kunz, Boston University. Concentrations of mercury (Hg) and methylmercury (MeHg) in biota from the northeastern United States exist because of atmospheric deposition and anthropogenic point sources. When Hg is methylated it becomes particularly toxic in ecosystem and to the wildlife. This paper examines Hg concentrations found in bat tissues over a four year period in the northeastern United States, and assesses differences sampled near point sources and from nonpoint sources. Percentages of MeHg found in total fur Hg were also evaluated to determine the level of toxicity commonly present in bat tissues. As predicted, Hg was significantly higher in bats captured near point sources compared to nonpoint sources. MeHg made up 86% (range = 71-95%) of the total Hg in 20 bats sampled in northeastern North America where several species are facing threats from white-nose syndrome (WNS), wind-power production, habitat loss, and pollution in air and water. MeHg is a widespread pollutant, and thus it is clear from the present study that a better understanding is needed regarding the neurological and behavioral effects of Hg on bats.

**MP217 Mercury trends in herring gull eggs from Atlantic Canada, 1972-2008** N.M Burgess, Environment Canada / Science & Technology Branch, Science & Technology Branch / Environment Canada, Canadian Wildlife Services / Environment Canada; A. Bond, University of Saskatchewan / Dept. of Biology; C.E. Hebert, Environment Canada / Science and Technology Branch, Ecotoxicology and Wildlife Health Division; L. Champoux, Environment Canada / SCIENCE AND TECHNOLOGY BRANCH; E. Neugebauer, Environment Canada / Science & Technology Branch. Mercury (Hg) is a pervasive contaminant that can adversely affect predatory wildlife. Bird eggs provide insights into breeding females' Hg burdens, and are easily collected and archived. We present data on Hg trends in herring gull (*Larus argentatus*) eggs from five sites in Atlantic Canada from 1972-2008. We found a significant decrease in Hg at Manawagonish Island, New Brunswick and Île du Corossol, Quebec, but after correcting Hg for dietary shifts using stable isotopes ( $\delta^{15}\text{N}$ ), these trends disappeared. Decreasing temporal trends of stable isotopes in gull eggs were observed at four sites, suggesting shifts in gull diets. At Gull Island, Newfoundland, diet-adjusted Hg increased from 1977-1992, dropped sharply between 1992 and 1996, and rose again from 1996-2008. After adjusting Hg trends for dietary shifts of herring gulls, it appears that environmental Hg in coastal ecosystems has remained relatively constant at most sites in Atlantic Canada over the last 36 years.

**MP218 Methylmercury Effects on Reproduction and Development in Breeding Homing Pigeons (*Columba livia*): A Study of Four Successive Breeding Rounds** J.K Moye, C. Perez, University of Nevada, Reno / Department of Agriculture, Nutrition and Veterinary Sciences; C. Pritsos, University of Nevada / Dept of Agriculture, Nutrition and Veterinary Science. Mercury is a heavy metal that has become a common contaminant in ecosystems throughout the world. An extensive number of studies have detailed bioaccumulation, and effects on birds have revealed the potential for mercury to adversely impact avian physiology and reproduction. There is concern that chronic exposure to toxicants at levels insufficient to cause overt mortality or other acute effects may have profound consequences for exposed individuals by means of increased reproductive dysfunction. This study was designed to investigate the impact of sub-lethal exposures to methylmercury on breeding homing pigeons (*Columba livia*) and assess reproductive and developmental effects upon offspring produced from sequential rounds of breeding. Homing pigeons were utilized as a surrogate species for other free-living birds. Breeding pigeons as well as offspring produced were exposed to 7.0 mg/L methylmercury via the drinking water, and a number of reproductive and developmental parameters were assessed over the course of four successive breeding rounds. Fertility, survival, and reproductive success were reduced in the exposed group for all four rounds while rates in controls remained at or near 100 percent. Significant differences

were also apparent when comparing days between clutches of exposed and control breeding pairs. Increases in days to fledge occurred in the exposed offspring and escalated from each round to the next. Chick weights were collected from hatching through 7 weeks of age, but resulted in no apparent differences between treatment groups. Results suggest that prolonged, sub-lethal exposure may have detrimental effects on reproduction and development and may be heightened through successive breeding rounds in avian species. Delays seen in initiation of sequential clutches may also have population level effects. These studies were funded in part by USGS and the Nevada Agricultural Experiment Station.

**MP219 Passerine Acute Oral Dose Study Update** K. Brugger, DuPont Crop Protection; M. Christ, Bayer CropScience; T.B. Fredricks, Monsanto / Zoology, Monsanto Company / Ecotoxicology & Ecological Risk Assessment; S.R. Mortensen, BASF Corporation; J. Overmyer, Syngenta Crop Protection, LLC / Ecological Sciences, Americas; P. Edwards, Syngenta / Environmental Safety. In 2007, the avian acute oral toxicity (LD<sub>50</sub>) study requirement for passerine species was added to the 40 CFR Part 158 Regulations without a standard protocol or test species. In some cases, the requirement may be waived (i.e. not required) if a large margin of safety is determined for the avian risk assessment based on quail or duck toxicity data and knowledge of the chemical being evaluated. To fulfill the requirement, registrants worked independently to initiate protocol development, standardize species selection, reduce regurgitation response, optimize dose administration methods, and evaluate how results of this study inform the avian risk assessment. CropLife America (CLA) member companies have since addressed these topics with the United States Environmental Protection Agency (USEPA) Office of Pesticide Programs (OPP) resulting in collective decisions for a path forward. As a result, passerine-specific protocol development progressed concerning species selection, fasting periods, and observation frequency. Regurgitation occurs in passerine LD<sub>50</sub> studies and can be a chemical-specific response leading to an underestimate of exposure and an invalid study. To overcome these issues a dietary toxicity (LC<sub>50</sub>) study using passerine species was proposed, but presents further challenges including starvation in response to food avoidance, as well as associated animal welfare issues and the absence of an agreed protocol. The purpose of the passerine study requires clarification. Does it represent an additional acute oral study or a means to compare toxicity between phylogenetically different species (i.e. with quail or ducks)? In either case, challenges are great with passerine studies either due to regurgitation or avoidance and starvation. Progress has been made by collecting data from more than 100 acute oral passerine toxicity studies indicating that sensitivity of passerines to an active ingredient is generally not greater than that for standard species. In these analyses, most end-points were within the same hazard category as previously tested birds and few end-points shifted from one hazard category to the next (e.g., practically non-toxic to slightly toxic). Regulatory impacts of these studies are considered in light of USEPA OPP methods for risk assessments.

**MP220 Physiological and Functional Effects of Aroclor 1254 on Avian Cognition and Migratory Behaviour** L. Flahr, University of Saskatchewan / Toxicology Centre; P. Jones, University of Saskatchewan / School of Environment and Sustainability, University of Saskatchewan / School of Environment and Sustainability and Toxicology Center; C. Morrissey, University of Saskatchewan / Biology. There is an increasing level of concern regarding endocrine disruption by a number of chemical pollutants that are widespread in the environment and commonly found in the tissues of wildlife. Birds exposed to endocrine disrupting chemicals during early development may develop neurological changes potentially affecting learning, cognition, and migratory behaviour. This study focuses on effects on the hypothalamic-pituitary-thyroid axis (HPT) in European starlings (*Sturnus vulgaris*) following sublethal oral exposure to Aroclor 1254, a PCB mixture, during early development. Alterations in the HPT axis could result in impaired thyroid function and physiological responses which could lead to behavioural changes in adults. Birds were orally administered 0, 0.35, 0.70, or 1.05 µg Aroclor 1254/g-bw for 18 days after hatch (day 1 to 18). Morphometric measurements and blood were taken to assess thyroid function during the developmental period. At the end of the nestling period, liver samples and feathers of euthanized birds were analyzed for total PCB content using gas chromatography/high resolution mass spectrometry and ethoxyresorufin O-deethylase (EROD) activity measured in the liver tissue. A subset of birds were reared in captivity and exposed to two photoperiod

shifts to simulate autumn and spring migrations as juveniles. Birds were observed for migratory activity and restlessness, and furcular fat, moult scores, and body mass measures taken twice weekly. Serial blood samples were taken (pre-migratory period, middle and completion of photoperiod shift) to assess thyroid function and status for each dose group. Emlen funnel trials were used to assess migratory orientation. This study is attempting to link alterations in avian behaviour to contaminant-specific mechanisms. Alterations in thyroid hormones could give rise to larger-scale effects, including changes in cognition and migratory behaviour, which may explain observed global declines in many migratory species.

**MP221 Phytic Acid Synthesis as a Potentially Inducible Response to Cadmium Exposure in Soybean Cultivars Differing in Cadmium Accumulation and Distribution** T. Jennett, University of Guelph / school of environmental science; B. Hale, University of Guelph / School of Environmental Sciences. Phytic acid (inositol hexakisphosphate) is an insoluble, indigestible phosphorus-rich molecule, and is the main storage form of phosphorus in seed tissue. Once synthesized, the acid is able to bind most cations, nutritionally important and otherwise, producing a phytate salt. These cations may then be unavailable for metabolic and enzymatic digestion in non-ruminant animals. Soybean cultivars OAC Bayfield (higher Cd-accumulating) and OAC Champion (lower Cd-accumulating), were grown in a hydroponic nutrient solution amended with Cd, and were tested for differences in phytic acid concentration at plant maturity (seed fill). Phytic acid/phytate was 15% greater in OAC Bayfield than OAC Champion, at both 5 and 20  $\mu\text{g Cd L}^{-1}$  nutrient solution. There was no difference in phytate between Cd exposures within cultivar, which suggests that this response may be, in part, genetically controlled, rather than an inducible response to Cd exposure. However, the coefficient of variation in these data was high, suggesting that a trend to greater concentrations of phytic acid in response to greater Cd exposure might be significant, with greater experimental power. If phytic acid is inducible in response to Cd, and reduces bioavailability to consumers of the grains, then this would be a significant modifier of potential toxicity.

**MP222 Polybrominated Diphenyl Ethers in Wisconsin River Otters and Michigan Bald Eagles** P. Dornbos, University of Michigan; S. Strom, Wisconsin Department of Natural Resources; T. Cooley, Michigan Department of Natural Resources; S. Chernyak, S. Batterman, J. Rutkiewicz, University of Michigan; N. Basu, University of Michigan / Department of Environmental Health Sciences, University of Michigan / Department of Environmental Health. Polybrominated diphenyl ethers (PBDEs) are commonly used as flame-retardants for a diverse array of commercial products including textiles, polyurethane foam, plastics, wood, and electronic material. PBDEs, structurally similar to polychlorinated biphenyls (PCBs), are an emerging chemical of toxicological concern especially to upper-trophic wildlife. Here, we assessed PBDE levels in two wildlife indicators from the Great Lakes – Wisconsin river otters and Michigan bald eagles. River otters ( $n=35$ ) were collected throughout the state of Wisconsin as part of their regular state-wide monitoring program, and liver tissues were used to assess levels of four PBDEs (BDE 47, 99, 100, and 153). All congeners were detected in all animals. The mean sum of the congeners was  $16.3 \pm 16.4$  ng/g, ranging from 0.5 to 72.9. The most common congener, accounting for 57.1% of the total, was BDE-47. BDE-47 averaged  $9.3 \pm 12.0$  ng/g and was significantly higher than the other congeners measured ( $P \leq 0.003$ ). In decreasing order, the remaining congener levels were BDE-99 ( $2.9 \pm 2.6$  ng/g), BDE-100 ( $2.7 \pm 3.4$  ng/g), and BDE-153 ( $1.4 \pm 2.3$  ng/g), which accounted for 17.8%, 16.6%, and 8.6% of the total, respectively. PBDE levels were not associated with sex or region collected. Similar data will also be presented from 35 bald eagles sampled from Michigan. Although much of the pentabrominated congeners production has been phased out, our study indicates detectable levels of four pentabrominated congeners in piscivorous wildlife in the Great Lakes.

**MP223 Predictive Modelling to Estimate the Accumulation of Soil-Borne Trace Elements in Field Grown Soybeans** T. Jennett, University of Guelph / school of environmental science; M. Morrison, Agriculture and Agri-Food Canada; B. Hale, University of Guelph / School of Environmental Sciences. Trace element contamination in crops and foodstuffs is becoming an issue of concern for many importing and exporting markets. Though refusal of imported crops has been minimal, concerns have been raised about an increasing trend in trace element accumulation in many

staple crops, including soybean, rice, sugar beet and grains. Soil and soybean seed samples were collected from the Ridgetown, Ontario area in a collaborative regional survey of trace element contamination by Agriculture and Agri-Food Canada and the University of Guelph. Ten soybean cultivars, exhibiting a range of Cd accumulation and distribution patterns (categorized as 'lower' and 'higher' accumulating cultivars, depending on breeding trials, and for classification in predictive models) were sampled from 78 local farming fields ( $n = 251$ ). Total seed and total soil [Cd] was measured using a hot Teflon bomb, concentrated  $\text{HNO}_3$  digestion. Soil solution pH was measured using 0.01 M  $\text{CaCl}_2$ . When regressed, soil pH and [Cd] were poor predictors ( $R^2 < 0.4$ ) of total seed [Cd] for most cultivars, suggesting that additional explanatory variables, such as 0.01 M  $\text{CaCl}_2$ -extractable Cd, soil composition or climatic data are necessary components for holistic, predictive models. Total and 0.01 M  $\text{CaCl}_2$ -extractable soil concentrations of Al, B, Co, Ni, Se and Zn were measured using ICP-OES or AAS for incorporation in predictive models as potential competing ions for Cd uptake and accumulation in plants. These trace elements are considered to be contamination concerns and are becoming increasingly scrutinized in international markets, and thus, are starting to be regulated.

**MP224 Riparian Arachnid Tactics for Managing PCB Body Burden** D. Delach, Clemson University / Environmental Toxicology; C.M. Lee, Clemson University / Environmental Engineering and Earth Sciences Department, Clemson University / Environmental Engineering and Science Department. Polychlorinated biphenyls (PCBs) contaminate the sediment of the Twelvemile Creek / Lake Hartwell Superfund Site, and are known to be transported throughout the resident biota via trophic transport. Riparian spiders have recently become of interest since not only are the aquatic biota found to have significant PCB body burdens, but also terrestrial organisms. Riparian spiders that consume primarily aquatic insects mediate this transfer, and can have a body burdens as high as 2400 ng/g w.w. Emergent insects carry contamination subsidies out of the river and into the riparian zone where they are captured by spiders, which effectively directs the contamination towards arachnivoracious predators such as lizards, frogs, and birds. *Tetragnathidae* spiders were collected along Twelvemile Creek, their enzymes isolated and exposed to individual planar and non-planar PCB congeners to assess their capacity for PCB metabolism. Mass spectroscopy has provided structural evidence that spiders can transform their body burden of PCBs to OH-PCBs for congeners with six or fewer chlorines. Additionally, spectra from a survey of the same species along the Twelvemile Creek arm of Lake Hartwell confirms that OH-PCBs are present in whole body extracts of spiders. Secondly, webs are hypothesized to play a protective role in spider ecotoxicology. Tetragnathid spiders are able to recycle approximately 90% of their web material without metabolizing it, thus creating an opportunity for web material to act as a storage location external to the body. Concentrations in webs ranged between 250 and 650 ppb. The enantiomeric fraction was also analyzed for chiral congeners to determine if storage in webs is an enantioselective process. Preliminary results indicate that the process is enantioselective.

**MP225 Selected organophosphate pesticide isolation, identification and determination in the feet of migratory songbirds** H. Alharbi, Carleton University / Department of Biology; P. Mineau, R. Letcher, Environment Canada / Ecotoxicology and Wildlife Health Division, Science and Technology Branch. Recent modeling analysis indicates that numerous birds are at risk of acute poisoning, and are routinely killed in large numbers in insecticide-treated fields. Although the majority of avian pesticide field studies have focused on the ingestion of treated seeds or insects (oral exposure), dermal exposure remains an important route of contamination when birds come into contact with atmospherically deposited pesticides or pesticide spill on foliage. Some migratory songbirds are particularly likely to encounter pesticide formulations as their non-breeding habitats are mainly crops or farmlands. Although feet as a route of exposure and as a matrix for analysis have received scarce attention in wildlife exposure studies, there are sufficient evidence and reports to show that feet can be a suitable matrix and indicator of dermal exposure to pesticide spillage or residue on foliage and the surface of other vegetation. The present work was primarily conducted to design, develop and evaluate a simple method of extraction and clean-up, as well as evaluate the potential of sensitive LC-QqQ/MS/MS-based analytical method for the quantitative analyses of selected OPPs at trace levels in feet of dead birds, and to use LC/TOF-MS, and LC-QTOF-MS/MS for confirmation of the positive findings. The developed method was then applied to

real samples of migratory songbirds that were opportunistically sampled during the spring in 2007 and 2011 in downtown Toronto, Canada, during the migration to their breeding sites. Only the feet of dead migratory songbirds were used for chemical analysis, where the assumption (hypothesis) was that migratory songbirds had been exposed via their feet to OPPs while foraging on pesticide-treated crops in the wintering habitat; and they retain OPPs residues on their feet, which can be quantitatively determined analytically. Among all the selected OPPs in this study, only chlorpyrifos residues were detected (which ranged from 0.5-1.2  $\mu\text{g mg}^{-1}$  feet.weight) in some species, these being Black throated blue warbler, Tennessee warbler, Northern parula, Northern water thrush, Common yellowthroat and Blue winged warbler. This study hypothesis was shown to be true as one of the OPPs included in the chemical analysis, i.e. chlorpyrifos, was detected and confirmed in the feet of some migratory songbirds. Furthermore, the feet of migratory songbirds were proven useful as a matrix to monitor dermal exposure of farmland birds to OPPs application to several crops. *OPPs: Organophosphate pesticides*

**MP226 Significant ecological effects of chronic exposure to the herbicide trifluralin and high acute toxicity of its formulation to larval amphibians** *S.M Weir*, The institute of environmental and human health / The institute of environmental and human health, The institute of environmental and human health / Environmental Toxicology; S. Yu, Texas Tech University / Department of Environmental Toxicology; C.J. Salice, Texas Tech University / Environmental Toxicology, Texas Tech University / TIEHH, Texas Tech University / Department of Environmental Toxicology. Fewer herbicides have been investigated for amphibian toxicity than insecticides, despite higher application rates for herbicides. For example, in 2007 more than 2.5 times the mass of herbicides was applied to Texas upland cotton than insecticides. In addition, herbicides were applied to 96% of all cotton acreage compared to only 43% for insecticides. Of the few herbicides investigated, atrazine and glyphosate (active ingredient in Roundup) have received the greatest research interest. We performed acute toxicity tests (96 hr) with 2 herbicides that have received little attention in amphibian ecotoxicology: trifluralin (sold as Treflan 4D) and pendimethalin (sold as Prowl 400 EC) and compared acute toxicity to an insecticide (malathion, formulated) and technical grade trifluralin. We used two tadpole species to represent eastern (green frog) and arid western (New Mexico spadefoot toad) anuran species. We also performed chronic toxicity tests with technical grade trifluralin on green frog larvae. Chronic toxicity treatments consisted of controls, an environmentally relevant concentration of 20  $\mu\text{g/L}$  and a higher (but still environmentally relevant) concentration of 200  $\mu\text{g/L}$  for 62 days. Both Treflan 4D and Prowl 400EC were significantly more toxic to green frog tadpoles than either malathion or technical grade trifluralin. Treflan 4D was also significantly more toxic than technical grade trifluralin to spadefoot toad tadpoles. Preliminary results indicated gut coil malformations in spadefoot toads exposed to technical grade trifluralin. Chronic exposure to pure trifluralin resulted in significantly smaller green frog tadpoles at 20  $\mu\text{g/L}$  compared to controls and 200  $\mu\text{g/L}$  treatments. Acute toxicity results suggest that many herbicides may have formulations that are more toxic to amphibians than the active ingredient or even some insecticides. The LC50s reported here are likely environmentally unrealistic at label rate specifications. Chronic toxicity results suggest a "non-monotonic" response of amphibians to trifluralin, but the mechanism is not known. Herbicides are applied at greater rates and are often detected more frequently in surface waters than insecticides, but very few herbicides (or their formulations) have been tested for toxicity to amphibians. Considering the toxicity found in this study and previous research into glyphosate formulations, more amphibian herbicide toxicity research is warranted.

**MP227 Splitting hairs: a forensic approach to characterizing grizzly bear exposure to metals using laser ablation – ICP/MS** *J. Christensen*, Stantec Consulting Ltd. / Toxicology; J. Spence, University of Victoria; K. Harris, Stantec Consulting Ltd.; C. Darimont, University of Victoria; P.S. Ross, Fisheries and Oceans Canada / Institute of Ocean Sciences. Stable isotope analysis of hair is frequently used by wildlife biologists to help characterize mammalian diets, since hair is metabolically inert and can record this dietary information chronologically. Like stable isotopes, metals obtained through diet are readily incorporated into hair, and once bound, remain relatively unchanged. Thus, a single grizzly bear hair provides a chronological account of metal exposure during the feeding season. Laser ablation –inductively coupled plasma mass spectrometry (LA – ICP/MS) was used to determine temporal changes in exposure to metals in grizzly bears from across British

Columbia. Samples were collected from hunted and management bears, as well as through hair snag programs. The main objectives of this project were to standardize this LA-ICP/MS technique for applications in metal biomonitoring programs for mammals, and then apply the technique to wild grizzly bears to characterize the influence of geography, salmon-reliance, age, sex, and season on metal accumulation. Typically, a single bear hair produced >1,500 individual data points along the strand, each representing a metal concentration. Metal concentration chronology starts at the tip (spring diet, emergence from hibernation) and ends at the root (fall diet, preparation for hibernation). Each bear had a distinct metal fingerprint that showed high reproducibility among replicates (i.e. very little variation in pattern or concentration among different hairs taken from the same bear). Seasonal patterns were evident in both interior and coastal grizzly bears. For coastal bears with access to salmon, there was increased dietary exposure to some metals, particularly copper and mercury, in the fall when salmon formed an important part of their diet. For interior bears, lead concentrations were high in the spring and decreased over time, suggesting a terrestrial/interior source of lead to these bears, and possibly a release of lead from internal stores during hibernation. While a traditional approach to metal analysis of a hair sample would yield only one measurement, representing eight months of cumulative exposure, our approach here provides a means to discern metal exposure in grizzly bears every 2-4 hours. This tool represents a potentially useful addition to wildlife biomonitoring programs and could be applied to a range of species.

**MP228 The Effectiveness of Using the Gulf Savers® Bag for *Spartina alterniflora* in Marsh Restoration at Popcorn Beach, Pass a Loutre WMA, Venice, Louisiana** *L.D Malizzi*, Matrix New World Engineering, Inc.; L. Carrere, M. Marshall, P. Marshall, Restore the Earth Foundation, Inc.; M. Moss, A. Parsons, S. Domangue, Matrix New World Engineering, Inc.. The use of the Gulf Savers® bag (bag) for *Spartina alterniflora* marsh restoration at Popcorn Beach at the Pass a Loutre WMA, Venice, Louisiana demonstrates the effectiveness of this approach for marsh restoration. The bag is a biodegradable, self-contained package of native plants with its own site-specific custom mixed supply of natural nutrients to support, feed, and protect the native vegetation. Each bag contains three, one year old *Spartina* seedling plugs and are the size of a sandbag (45 cm). The bag is a stability kit that jump starts growth and survivability in the face of storm surge, wave action, and rapid erosion. As one of the three principal outlets of the Mississippi River, the Pass a Loutre Site is challenging to restore using traditional planting techniques due to the variable tidal range, high wave energy and substantial discharge rates on-site creating an obstacle to *Spartina* plug establishment. In December 2010, 400 bags (1200 plugs) were placed on the north end of Popcorn Beach as a demonstration project. Half of the bags (200 bags) were distributed in cluster formation (2-3 bags) in a checkerboard pattern, in an approximately 22 m by 25 m area adjacent to the beach, with 3 m between bag clusters. The remaining half (200 bags), were deployed as a continuous row along portions of the perimeter in order to enhance sediment capture and to buffer interior bag groupings from high energy tidal forces. In March of 2011, 50 additional bags of *Spartina* plugs in cluster formations were deployed to reinforce the perimeter by arraying bags adjacent to the perimeter rows deployed in December 2010. The first monitoring event at the Site was conducted in December of 2011 at pre-surveyed, permanent 1 m by 1 m plots. The average percent cover of the *Spartina* was 78%, with an average canopy height of 93 cm in the bag area. A second monitoring was conducted at the same plot locations in April of 2012. The average percent cover was 85% and the average canopy height was 119 cm in the bag area. Of importance is the fact that the *Spartina* had completely filled in the treatment area in a twelve month period. In addition, the initial linear formation of *Spartina* along the perimeter had expanded outwards to a width of 8 m from the original planting in 17 months. The percent cover, average canopy height, and aerial extent of the *Spartina* demonstrates the effectiveness of the bags for use in marsh restoration.

**MP229 The Effects of Maternally Transferred Methylmercury on Leukocyte Differentials in Northern Water Snake (*Nerodia sipedon*) Neonates** *V. Kremer*, Middle Tennessee State University / Department of Biology; C. Henry, R.C. Wright, Middle Tennessee State University / Biology; P.W. Cusaac, V. Cobb, M. Klukowski, F.C. Bailey, Middle Tennessee State University / Department of Biology. The ability to mount a response to stress is essential to the survival of any organism. Although the effects of mercury on



stress in vertebrates have been well documented, little research in this area has been done on reptiles. Mercury is a common contaminant in aquatic systems, and tends to accumulate in predators at the top of the food chain, like the Northern Water Snake (*Nerodia sipedon*). It is known that stress influences leukocyte ratios in vertebrates, and fluctuations in these ratios can be used to assess stress levels. This study examines the effects of maternally transferred methylmercury on stress in *N. sipedon* neonates using leukocyte ratios, and additionally provides baseline leukocyte parameters for *N. sipedon* neonates, which have not been previously reported. 18 gravid females were collected at Lake Erie and dosed in the laboratory at Middle Tennessee State University with one of three concentrations of methylmercury (0, .01, and 10 µg/g body mass.) After birth, peripheral blood samples were taken from 10 neonates from each litter and smears were prepared using a Giemsa stain. The smears were analyzed under by light microscope, and leukocyte differentials were recorded for each neonate. Preliminary results suggest no significant difference in leukocyte parameters between mercury doses; however, an unexpected inverse relationship was discovered between basophil and lymphocyte ( $r = -.7869$ ,  $p = 0.002$ ) as well as heterophil and lymphocyte ( $r = -.5847$ ,  $p = 0.046$ ) numbers in neonates.

**MP230 The Effects of Methoxychlor on Reproduction and Behavior of the California Vole, *Microtus californicus*** N. Amundson, University of California at Davis; M. Johnson, John Muir Institute of the Environment, UC Davis, and Michael L. Johnson LLC. Methoxychlor is a chlorinated pesticide that can function as an estrogen agonist in exposed rodents. The California vole, *Microtus californicus*, is an herbivorous rodent that mediates plant composition and structure in grasslands and is an important prey species across habitats throughout California. The results of our multi-generation laboratory study of the effects of methoxychlor on reproduction in *M. californicus* determined that effects varied depending upon exposure route and dose. For the directly dosed parental (F0) generation, the response was non-monotonic, with productivity significantly stimulated in voles at the low dose relative to the control and high dose exposure concentrations. Productivity of the *in utero*/lactation exposed offspring also differed between the treatment and control groups, and was due to a higher ratio of live to total pups. Fertility in the parental cohort was significantly reduced at the high dose but did not differ between the control and low dose exposure groups and did not differ across treatments for the F1 generation. The effect on fertility in the high dose group was reversible and productivity increased post-exposure. Offspring sex ratio was not affected by methoxychlor for the parental generation, but was significantly skewed toward female in the offspring of the *in utero*/lactation exposed F1 generation, indicating the possibility of an epigenetic alteration to the germ line in the F1 parents. Methoxychlor exposure also resulted in effects on hormonally mediated behaviors in *M. californicus*. Infanticide was significantly reduced in both parental (F0) and F1 generation voles. The effect on the directly dosed adult dams is postulated to be due to estrogen receptor binding and associated stimulation of oxytocin production, whereas reduced infanticide in the *in utero*/lactation exposed F1 generation is hypothesized to be due to epigenetic alteration. Running wheel activity by methoxychlor-exposed F1 generation dams was increased compared with controls, while territorial behavior in F1 generation treated and control males did not differ. Long term alterations to sex ratio coupled with effects on hormone mediated behaviors that regulate the social systems of *M. californicus* could result in impacts to vole population dynamics in natural habitats.

**MP231 The effects of the insecticide p,p'-DDT and p,p'-DDE on androgen receptor signalling in the human LNCaP prostate cancer cells** L.I. Wong, Simon Fraser University / Department of Biological Sciences; J. Elliott, Environment Canada / Pacific Wildlife Research Centre, Science and Technology Branch, Environment Canada / Pacific Wildlife Research Centre, Environment Canada / Wildlife Toxicology, Environment Canada / Canadian Wildlife Service; T.V. Beischlag, Simon Fraser University / Faculty of Health Sciences. The legacy insecticide DDT (1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane) and its main metabolite, DDE (1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene) are well recognized as endocrine disruptors. Despite its ban, DDT and DDE still continue to persist in the environment and in biota. o,p'-DDT and p,p'-DDE have been shown to act as potent estrogen receptor (ER) agonists and androgen receptor (AR) antagonists, respectively whereas p,p'-DDT (the more common isomer) is a weak ER agonist and AR antagonist. In this study, we investigated the effects of p,p'-DDT and p,p'-DDE on mRNA and protein accumulation of

AR, and the AR target genes, prostate-specific antigen (PSA) and prostate-specific membrane antigen (PSMA) in the human prostate cancer cell line, LNCaP. Environmentally relevant concentrations of 1 µM and 10 µM were chosen for the two chemicals. We have demonstrated that mRNA accumulation of PSA is lower than basal levels when treated with p,p'-DDE at 10 µM and p,p'-DDT at both concentrations. In combination with the synthetic androgen, R1881, PSA expression is repressed by p,p'-DDT and p,p'-DDE. At the mRNA level, both chemicals alone at 10 µM and in combination with R1881 relieved the R1881-inducible repression of PSMA expression. At the protein level, both chemicals at both concentrations alone and in combination with R1881 significantly relieved the R1881-inducible repression of PSMA, which was inconsistent with our mRNA accumulation data. Furthermore, we found that p,p'-DDT and p,p'-DDE at 10 µM repressed R1881-inducible AR protein accumulation. Collectively, these results demonstrate that these chemicals may not have the same properties as classic agonists of the AR but may affect AR levels by up-regulation. This is significant as the endocrine disruption effects of DDT and DDE include reproductive and developmental abnormalities in wildlife and humans and may be linked to hormone-dependent cancers.

**MP232 The influence of copper sulfate on life-history parameters of the collembolan, *Sinella curviseta*** J.B. Schmidt, Roskilde University / ENSPAC; D.M. Jevtic, Jagiellonian University / Institute of Environmental Sciences; A. Palmqvist, Roskilde University / Environmental, Social and Spatial Change, Roskilde University / Dept. of Environmental, Social & Spatial Change; R. Laskowski, Jagiellonian University / Institute of Environmental Sciences; V.E. Forbes, University of Nebraska Lincoln / School of Biological Sciences. Collembolans are widespread all over the world and known to play an important role in soil ecosystems as decomposers of soil organic matter. For ecotoxicological testing *Folsomia candida* has prevailed as the standard organism of choice and is extensively used in experiments for the assessment of chemicals. Another OECD-recommended springtail species, *Sinella curviseta*, is much less studied. Although both species can be used in ecotoxicological tests, they differ substantially in life histories, the former being parthenogenetic, and the latter sexually reproducing. The importance of organism life-history for population-level effects of chemicals is widely recognized, but little empirical information can be found on this issue for collembolan species. Experimental approaches usually focus on survival and reproduction measured over short time periods and are likely to miss time-related effects essential for maintaining viable populations. In a series of studies on the *S. curviseta*, several life-history parameters were used to characterize the chain of effects leading to population-level responses caused by copper pollution. To examine the consequences of copper exposure in soil, a 10-week toxicity experiment was performed. Reproduction was clearly reduced in contaminated soils whereas population growth rates reached similar levels up to a concentration of 1500 mg/kg dw after 8 weeks of exposure. Due to practical difficulties in following all individuals in the soil matrix, a life table response experiment was conducted on plaster of Paris. However, results of this experiment were counterintuitive showing enhanced fecundity at higher Cu concentrations. Experiments on acute toxicity and egg survival over time as well as bioaccumulation of Cu indicated that *S. curviseta* can tolerate high concentrations. The results, challenges, and experimental designs for the characterization of additional life-cycle variables to gain insight into individual-level mechanisms leading to population-level consequences will be discussed. Our general conclusion is that *S. curviseta*, being an OECD-recommended test species, requires more in-depth studies on its resistance to specific groups of pollutants and the importance of its life-history to the observed effects. The study showed extremely high resistance of the species to copper and indicates that using *S. curviseta* as a standard test species may lead to underestimation of ecological risks, at least for some chemicals.

**MP233 The Michigan Bald Eagle Biosentinel Program: Long-term biomonitoring, past, present and future** L. Fuentes, Clemson University / Department of Forestry & Natural Resources; W. Bowerman, University of Maryland / Department of Environmental Science & Technology; W. Bridges, Clemson University / Department of Mathematical Sciences; J. Sikarskie, Michigan State University / College of Veterinary Medicine. The bald eagle (*Haliaeetus leucocephalus*) is an ideal sentinel species for environmental contaminants assessment because of the species' exposure potential, geographic occurrence, ease of sample collection, and quantity of existing data. The population productivity of the bald eagle in Michigan has been

monitored since 1961 following a precipitous decline in successful breeding attempts due to the presence of contaminants in the Great Lakes ecosystem. In 1999, the Surface Water Quality Division of the Michigan Department of Environmental Quality implemented a monitoring program using nestling bald eagles to monitor trends of persistent contaminants. These contaminants include organochlorine pesticides such as dichlorodiphenyl-trichloroethane (DDT), polychlorinated biphenyls (PCB) and mercury (Hg). Since the banning of DDT and PCB, the bald eagle population has been increasing from fewer than 80 active breeding areas to more than 650 throughout the state in 2012. These data correspond to the decline in OC and PCB concentrations in nestling blood samples below lowest-observable-adverse-effect-levels (LOAEL) in all areas with the exception of watersheds and shoreline areas with the highest historical contamination. Conversely, feather samples indicate that Hg is on the rise throughout the state of Michigan. With 51 years of productivity data, almost 15 years of blood and feathers samples, and biometric measurements, the Michigan bald eagle dataset can be used to answer questions about historical, persistent contaminants and metabolites, emerging contaminants of concern, and inquiries about the bird population and its associated aquatic food chain. Archived feather samples from nestlings that were noted as being sick or exhibiting abnormal behavior from 1999 to 2012 were run with inductively coupled plasma analysis to screen for possible observable toxicological effects from contaminants such as Hg and lead. When biometric data were examined between 1999 and 2012, trends suggested that nestling body size is decreasing which may be indicative of adaptations to anthropogenic stressors and/or natural changes in the environment such as climate and density-dependent effects of the growing population. Multiple stressors can present limitations in data interpretation, but with the ability to look to archived samples and data, long term monitoring datasets can assist with current monitoring efforts and lay groundwork for future studies.

**MP234 Time of Flight Effects in Homing Pigeon (*Columba livia*) Offspring Parentally and Directly Exposed to Methylmercury** J.K. Moyer, University of Nevada, Reno / Department of Agriculture, Nutrition, and Veterinary Sciences; C. Perez, University of Nevada, Reno; C. Pritsos, University of Nevada / Dept of Agriculture, Nutrition and Veterinary Science. Mercury is a globally distributed contaminant introduced in many ecosystems as discharge from human activities such as agriculture, industrial and mining. The organic form of mercury (methylmercury) is of the greatest concern due to its ability to bioaccumulate in animal tissue and concentrate in high levels at the top of the food chain. Among the most vulnerable species to exposure are birds that live, nest, or feed in or near methylmercury contaminated ecosystems. Conventional avian toxicology models have shown impairment of cognitive skills from exposure to levels higher than those generally considered environmentally relevant, but little work has been conducted on low-level exposure effects. Because of the known neurological effects of mercury on birds, it is hypothesized that effects upon migratory ability would be evident after exposure to low levels of this metal, and effects may be exacerbated in young birds. Difficulties in following mercury exposed birds once they migrate away from contaminated areas have left investigators with insufficient data to establish exposure levels causing injury of migratory species due to migration disruption. Homing pigeons (*Columba livia*) have been used as a model species to study avian orientation and navigation. It is suggested that the navigational system of wild birds is based on the same principles of the pigeon's ability to home. Thus, effects upon homing may be used as a surrogate for effects upon migratory ability in other species. Breeding pigeons were exposed to ~1.0 mg/kg/day methylmercury via the drinking water, and first round offspring were trained to home after fledging, while also continually exposed to methylmercury. The young pigeons were released individually for three flights, and flight times were assessed and compared to control young pigeon flight times from 3.5, 9, 21, 53, 65, and 98 air miles as well as two individual flights at ~50 air miles from multiple directions. Results indicate that methylmercury exposed birds exhibit slower flight times than controls during the initial flight, and generally improve on successive flights at each distance and direction. This may suggest orientation impairment and allude to migration disruption in migratory species. These studies were funded in part by USGS and the Nevada Agricultural Experiment Station.

**MP235 Twenty years of research and monitoring in the Great Blue Heron along the St. Lawrence River: Temporal trends of contaminant levels and effects** L. Champoux, Environment Canada / Science and

Technology Branch. In 1996, after many years of preliminary studies, the great blue heron (*Ardea herodias*) was selected as a sentinel species for monitoring the state of the St. Lawrence River and the Gulf of St. Lawrence in Québec, Canada. As part of this program, selected great blue heron colonies along the St. Lawrence River and its estuary were visited at five year intervals from 1991 to 2011, to collect eggs as well as blood samples from juveniles. Contaminant analyses were conducted at Environment Canada's National Wildlife Research Centre (Ottawa, Canada). Contaminants measured included mercury, PCBs, organochlorines, dioxins and furans, and brominated flame retardants (BFRs). In eggs, mercury showed a decreasing temporal trend only in the fluvial section of the river, while PCBs and most organochlorinated pesticides also showed significant decreases further down the estuary and Gulf of St. Lawrence. Although measured only more recently, the sum of BFRs also showed a significant decrease over time in eggs. Significant decreases over time were also observed in some contaminants in the blood and plasma from juvenile birds. Trends in biomarkers in eggs and in plasma were related to contaminant levels and potential toxicity.

**MP236 Uncertainty assessment of a one-compartment toxicokinetic model used in biomonitoring otter exposure to polychlorinated biphenyls** J. Trowell, Simon Fraser University, Azimuth Consulting Group, Simon Fraser University; N. Healey, P. Allard, Azimuth Consulting Group. Scat sampling and analysis has been used as a non-invasive biomonitoring method to assess otter exposure to polychlorinated biphenyls (PCBs). A one-compartment, first order toxicokinetic model published by de Vries (1989) has been used to estimate PCB concentrations in otter muscle from PCB concentrations measured in otter scat. Estimated PCB muscle concentrations are then, in turn, used to make inferences about the potential health risks to otters based on concentration-response relationships reported for mustelids. The objective of the presented work is a critical review of the toxicokinetic model to assess model uncertainty and its application in biomonitoring. Model uncertainty was investigated qualitatively by critically reviewing the range of plausible values for the model input parameters, and quantitatively by developing a probabilistic version of the toxicokinetic model to estimate the probable PCB muscle concentrations in otters based on contemporary measures of PCBs in the scat from Victoria Harbour, British Columbia, Canada. The results of the model uncertainty analysis support the following conclusions: (1) the default values for the model input parameters are reasonable and defensible point estimates; (2) the central tendency of the output of the probabilistic model is in good agreement with the output of the deterministic model; and (3) there is approximately a 15-fold difference between the 5<sup>th</sup> and 95<sup>th</sup> percentiles of the probability distribution of PCBs in otter muscle tissue (6 and 85 mg/kg lipid) for a given input of mean measured PCB concentration in otter scat of 10.6 mg/kg lipid. The predictive accuracy of the model could not be assessed as we were unable to identify any studies in the open literature that included synoptic measures of PCBs in otter scat and muscle tissue. The results of our investigation indicate a relatively high degree of uncertainty in the modelled relationship between PCB concentrations in otter scat and PCB concentrations in otter muscle tissue when examined in the context of the key sources of uncertainty and variability in a risk assessment for otter based on measurements of PCBs in scat.

**MP237 Utility of a Passerine Dietary Test (LC<sub>50</sub>) to Determine Toxicity of Pesticide Products that Elicit an Emetic Response in Standard LD<sub>50</sub> Testing** M. Christ, Bayer CropScience; T.B. Fredricks, Monsanto / Zoology, Monsanto Company / Ecotoxicology & Ecological Risk Assessment. The current LD<sub>50</sub> study design for passerine species testing can result in an undesirable regurgitation response from oral dosing and exposure to pesticide products that can cause an emetic response. Regulatory authorities have expressed concern over the regulatory interpretation of studies in which regurgitation is observed with the canary (*Serinus canaria*) or zebra finch (*Taeniopygia guttata*) from not receiving the complete oral dose. A dietary LC<sub>50</sub> study has been proposed as a potential test to determine dietary toxicity for passerine species when an acceptable LD<sub>50</sub> study can not be conducted. The current research provides information on the usefulness of a LC<sub>50</sub> dietary passerine test to assess the toxicity of two pesticide products on the canary. Both compounds were previously used in LD<sub>50</sub> trials and characterized as producing low toxicity values with a steep slope (MCPA Acid) and high toxicity values with a moderate slope (Isazofos). The birds were exposed to treated feed with MCPA Acid (herbicide) and Isazofos (insecticide) over a 5-day exposure period followed by a 3-day recovery period



with clean (untreated) feed. In an attempt to mimic a bolus dose that birds would receive during an oral dose test, greatly concentrated feed levels were prepared to achieve the proper daily dietary dose (mg ai/kg bw/day) based on pre-exposure individual dietary intakes. End-points determined for each trial included: sub-lethal effects, mortality, body weight, and food consumption. The passerine dietary test could prove as a useful study for assessing toxicity for compounds with emetic properties, however further ring-testing with additional chemicals will better determine the utility of this test design. Additionally, uncertainty about how to include a dietary passerine test into the current USEPA risk assessment strategy needs to be determined.

**MP238 Effects of Maternally Transferred Methylmercury Chloride on Oxidative Stress in *Nerodia sipedon* Neonates** P.W. Cusaac, F.C. Bailey, Middle Tennessee State University / Department of Biology. Chronic oxidative stress can lead to long term damage in major organ systems, such as the liver. Cause and response interactions of stressors are relatively well studied, however studies showing the effects of heavy metal toxicity on stress are lacking, particularly in squamate reptiles. The purpose of this study was to show the effects of maternally transferred methylmercury chloride on baseline elevation of oxidative stress in *Nerodia sipedon* neonates. Adult females were dosed with one of three dosing treatments (control, 0 ug/g, and 10 ug/g MeHgCl per gram of snake) and allowed to birth. Neonatal liver concentrations of Malondialdehyde (MDA, a free radical byproduct of polyunsaturated lipid peroxidation) were measured to quantify oxidative stress. Liver MDA levels differed significantly across doses ( $F_{(2,10)} = 5.320$ ,  $p = 0.034$ ), with post-hoc comparisons showing MDA levels in the 10 ug/g treatment as significantly less than the controls. It can therefore be concluded that MeHgCl does affect background oxidative stress in *N. sipedon* neonates.

**MP239 Effects of the Herbicide Imazapyr on Juvenile Oregon Spotted Frogs** A. Yahnke, Washington Cooperative Fish and Wildlife Research Unit, University of Washington / MS student, University of Washington, WACFWRU / PHD student; C. Grue, Washington Cooperative Fish and Wildlife Research Unit, University of Washington / School of Aquatic and Fishery Sciences, USGS, Washington Cooperative Fish and Wildlife Research Unit / University of Washington; M.P. Hayes, Habitat Program, Washington Department of Fish and Wildlife; A.T. Troiano, Washington Cooperative Fish and Wildlife Research Unit / School of Aquatic and Fishery Sciences, University of Washington, University of Washington / School of Aquatic and Fishery Sciences, University of Washington. Conflict between native amphibians and aquatic weed management in the Pacific Northwest is rarely recognized because most native stillwater-breeding amphibian species move upland during summer, when herbicide application to control weeds in aquatic habitats typically occurs. However, aquatic weed management may pose a risk for aquatic species present in wetlands through the summer, such as the Washington State Endangered Oregon Spotted Frog (OSF; *Rana pretiosa*). Acute toxicity of herbicides used to control aquatic weeds tends to be low, but the direct effects of herbicide tank mixes on OSF have remained unexamined. We exposed juvenile OSFs to tank mixes of the herbicide imazapyr, a surfactant, and marker dye in a 96-h static-renewal test. The tank mix was chosen because of its low toxicity to fishes and its effectiveness in aquatic weed control. Concentrations were those associated with low (3.5 L/ha) and high (7.0 L/ha) volume applications of imazapyr, and a clean-water control. Following exposure, frogs were reared for 2 mo in clean water to identify potential latent effects on growth. Endpoints evaluated included feeding behavior, growth, and body and liver condition indices. We recorded no mortalities and found no significant differences for any endpoint between the herbicide-exposed and clean-water control frogs. Results suggest that imazapyr use in wetland restoration poses low risk of direct toxic effects on juvenile Oregon Spotted Frogs.

**MP240 EROD activity in wild and laboratory birds with embryonic exposure to environmentally relevant PCB mixtures as an assessment of biomarkers of exposure** M.E. Barton, University of Maryland / Animal and Avian Sciences; K. Dean, University of Lethbridge / Neuroscience, University of Maryland / post-doctoral research fellow; M. Ottinger, University of Maryland / Department of Animal and Avian Sciences. Biomarkers of exposure to environmentally persistent organic compounds provide critical data for use in assessing potential risk to wild populations. Ethoxyresorufin-O-deethylase (EROD) activity has been used largely as a biomarker of dioxin exposure, but has also been used for assessing exposure of wildlife to dioxin-like compounds such as polychlorinated biphenyls (PCBs). PCBs can vary

in their ability to induce EROD, which is an important consideration in establishing EROD as a valuable biomarker for PCB exposure. We studied EROD activity in two experiments: one in wild birds (*Tachycineta bicolor* and *Sialia sialis*) and one in a laboratory avian model (*Coturnix japonica*) which our studies indicate has a similar sensitivity to that seen in some wild birds. Four compounds were administered to Japanese quail during embryonic development: two environmentally relevant PCB mixtures found at the upper Hudson River and two single congeners, PCB 126 and PCB 77. The wild bird study revealed a congener related difference in response. Embryonic exposure to PCB 77 resulted in a relatively flat response in tree swallows, while the two PCB mixtures and PCB 126 yielded clear increasing trends in response profiles. The laboratory model study showed similar responses. These data provide evidence that PCB 77 may not have strong dioxin-like biological activity in some avian species, which is in agreement with a previous WHO report that has proposed a recalculation of the PCB 77 TEF from 0.0005 to 0.0001. As such, indices of toxicity such as TEQs and TEFs should be utilized to estimate potential effects, with the recognition that these calculations may not be fully informative for all mechanisms of toxicity, including endocrine disruption and other non-aryl hydrocarbon receptor mediated actions. These data also contribute to our understanding of EROD response to complex PCB mixtures. The conclusions and opinions presented here are those of the authors, they do not represent the official position of any of the funding agencies, the Hudson River Trustees, or the United States.

**MP241 Genomic effects of dietary exposure to 1,2,5,6,9,10-Hexabromocyclododecane (HBCD) in Japanese quail** N. Karouna-Renier, USGS Patuxent Wildlife Research Center / Beltsville Lab, U.S.G.S.-Patuxent Wildlife Res. Ctr / Beltsville Lab; C. Maddox, Patuxent Wildlife Research Center, USGS / Beltsville Lab; P.F. Henry, U.S. Geological Survey / Patuxent Wildlife Research Center; Y. Chen, Virginia Tech / Department of Fisheries and Wildlife Sciences, USGS Patuxent Wildlife Research Center, USGS Patuxent Wildlife Research Center / Patuxent Wildlife Research Center, U. S. Geological Survey / Patuxent Wildlife Research Center; D. Sprague, J.A. Green, USGS Patuxent Wildlife Research Center. 1,2,5,6,9,10-Hexabromocyclododecane (HBCD) is a globally distributed, high production volume brominated flame retardant, which has demonstrated endocrine effects related to the thyroid, progesterone, estrogen, and androgen systems. Toxicity concerns surrounding HBCD stem from its persistence, potential for long-range transport in the environment, evidence of its bioaccumulation and biomagnification in the food chain, and potential reproductive, developmental, and neurological effects in terrestrial and aquatic species. The current study investigated the effects of HBCD on gene expression in Japanese quail (*Coturnix japonica*) continuously exposed through feed to one of five doses of HBCD for nine weeks beginning at week six of age. Gene expression indicative of potential endocrine disrupting effects including sex-steroid responsive genes, thyroid hormone related genes, and biotransformation enzyme-related genes, was analyzed in adult quail tissues using real-time quantitative PCR. Results across treatment groups were compared to evaluate dose-related effects of HBCD exposure. These data were collected from the first generation of a multiple generation study of HBCD and represent the effects of HBCD on birds exposed only through feed.

**MP242 Impact Assessment of Gopher Getter, a Rodenticide Containing Strychnine, on Great Basin Gophersnakes (*Pituophis Catenifer Desertiicola*)** K. WILLIAMS, Van Hees Environmental Consultants; C. Bishop, Environment Canada / SCIENCE AND TECHNOLOGY BRANCH, Environment Canada / Wildlife and Landscape Science, Environment Canada / Canadian Wildlife Service; D. KIRK, Aquila Conservation & Environment Consulting; J. Elliott, Environment Canada / Pacific Wildlife Research Centre, Science and Technology Branch, Environment Canada / Pacific Wildlife Research Centre, Environment Canada / Wildlife Toxicology, Environment Canada / Canadian Wildlife Service. Vineyards and orchards currently represent a significant proportion of the land cover in the Okanagan valley BC. Rodents, such as pocket gophers (*Thomomys talpoides*), eat young roots and chew bark which can girdle the tree or vine. Strychnine, the active ingredient in the product Gopher Getter, is put down holes and tunnels to kill the pocket gophers and bring their populations back under control. Great Basin gophersnakes (*Pituophis catenifer deserticola*) are known to eat pocket gophers across their range. We created a model to estimate the exposure of gophersnakes to strychnine, incorporating for example, feeding frequency, prey composition and amount, body mass, and lethal doses of



strychnine for pocket gophers and gophersnakes. We evaluated dose at 2 levels, indicating the lowest and highest probable numbers of gophersnakes impacted by strychnine in the Okanagan Valley. We obtained various GIS layers to visualize the extent of the various factors involved, including TEM (terrestrial ecosystem mapping) maps of the south Okanagan and partial TEM maps of the central and north Okanagan, Ministry of Agriculture land use coverage of orchards and vineyards in the Okanagan south of the Peachland area, and a TEM gophersnake habitat suitability model. Data was obtained from the pesticide division of Ministry of environment in Penticton, BC, from vendors local to the south Okanagan. We obtained records for two years from 2005 – 2006 and averaged yearly data to obtain our yearly volume sold (1712 kg). We estimated that between 72 and 3392 gophersnakes could be poisoned per year by Strychnine exposure through consumption of poisoned pocket gopher prey.

**MP243 A Multi-Analyte Method for the Determination of Selected Current-Use and Legacy Pesticides, and PBDEs in Human Milk** S. Boone, U.S. EPA / Office of Chemical Safety and Pollution Prevention, USEPA/ECB / USEPA/OCSP/OPP/BEAD/Environmental Chemistry Laboratory; C. Vigo, T. Boone, C. Byrne, USEPA/OCSP/OPP/BEAD/Environmental Chemistry Laboratory; B. Guan, A2R; J. Ferrario, USEPA/OCSP/OPP/BEAD/Environmental Chemistry Laboratory. A multi-analyte method to determine selected current-use and legacy pesticides, and the predominant polybrominated diphenyl ethers (PBDEs) in human milk was developed at the Environmental Chemistry Branch to evaluate potential exposures to infants during breast feeding. The method uses a limited sample volume with minimal preparation time at relevant low detection limits. The compounds of interest in this method include: aldicarb, atrazine and metabolites (DEA, DIA, DACT), bifenthrin, carbaryl, chlorpropham, chlorpyrifos, cyfluthrin, cyhalothrin, cypermethrin, deltamethrin, p,p'-DDE, diazinon, diphenylamine, endosulfan ( $\alpha$ ,  $\beta$ ), endosulfan sulfate, fenvalerate, fipronil, hexachlorobenzene, imazalil, malathion, o-phenylphenol, PBDEs (47, 99, 100, 153, 154), pentachloronitrobenzene, permethrin (cis, trans), tau-fluvalinate, thiabendazole, and triclosan. Twelve surrogates are used in this method: the deuterated forms of atrazine, chlorpyrifos, trans-cypermethrin, and diazinon and the carbon labeled forms of carbaryl, p,p'-DDE, hexachlorobenzene, cis-permethrin, trans-permethrin, o-phenylphenol, thiabendazole, and triclosan. The extraction method is a modified QuEChERS method which includes the extraction of milk with acetonitrile followed by an initial C18 SPE column clean-up. Subsequent sample processing is determined based on the requirements of the instrumental analysis: GC/HRMS, GC/MSD-CI, or LC/MS/MS. All three use separate PSA SPE columns: elution with acetonitrile for LC/MS/MS, elution with acetone/toluene for GC/MSD-CI, and elution with acetone/toluene on a modified PSA SPE column with the addition of a neutral alumina top bed volume for GC/HRMS. Matrix matched calibration procedures are required for the analyses by GC/MSD and LC/MS/MS. A critical consideration in the effective use of these matrix-matched standards for trace-level analyses is the need to find relatively clean breast milk. The matrix effect of the human milk for certain pesticides (e.g., triclosan) is markedly different from that of other animal species. The LODs for the selected compounds range from 0.01-0.5 ppb based upon sensitivity, background, and matrix effects. Additional compounds tested for this method were removed for a number of reasons: 2,4-D (lack of isolation on PSA), acephate (split peaks at solvent front), and amitraz (lack of isolation on C18 and PSA).

**MP244 Carbon-based cleanup approach for DDT in sediments from the Palos Verdes Shelf** L. Scharpenberg, D. Tai, Calscience; K. Carlyon, ITSI. Calscience has incorporated a new cleanup approach to be used in conjunction with EPA 3660 for the reduction of sulfur and others matrix-based interferences, when analyzing WWTP effluent-affected sediment samples for DDT compounds, in support of the Palos Verdes Shelf project. When sample extracts are introduced into a hot GC injection port, DDT has a propensity to completely degrade into their lesser byproducts, DDE and DDD. This breakdown is exacerbated in the presence of sulfur and other matrix components that are co-extracted from sediments. At times a single sample injection can leave sufficient residue on the injection port liner to cause DDT breakdown to exceed the acceptance criteria for the method. This in turn leads to constant injection port maintenance, multiple re-runs, failing QC, potentially biased low DDT results, and qualified data. In order to minimize the effect on the liner, and thus minimize DDT breakdown, Calscience investigated the use of a carbon-based cleanup cartridge from

Supelco/Sigma Aldrich. The cartridge is a premanufactured dual-layer, solid phase extraction cartridge which consists of an ENVI-Carb layer (carbon) on top, and a PSA (primary and secondary amines) layer on the bottom. The combination has a strong affinity toward (can isolate/remove) pigments (e.g. chlorophyll and carotenoids), sterols, fatty and organics acids. In order to test this cleanup step, Calscience received two blind aliquots of a pre-dried Palos Verdes Shelf SRM. These aliquots were prepared, each in duplicate, using EPA 3545 and analyzed using EPA 8270C SIM for the following analytes: 2,4'-DDD, 2,4'-DDE, 2,4'-DDT, 4,4'-DDD, 4,4'-DDE, 4,4'-DDT, 4,4'-DDMU, and 4,4'-DDNU. Prior to analysis, one set of the duplicates was subjected to just the EPA 3660 cleanup procedure (using mercury), and the other was subjected to the EPA 3660 procedure plus the ENVI-Carb/PSA cleanup. The outcome of this study shows that the sample receiving only the standard sulfur cleanup exhibited results for the 4,4'-DDT that were between 5 and 20 times less than the dual cleanup step. Using the ENVI-Carb/PSA cartridge cleanup, the DDT analytical results agreed nicely with the SRM values. Surrogate and matrix spike recoveries were within criteria using the dual cleanup step. The relative percent difference for the 4,4'-DDT was less than 6% using the dual cleanup approach, and was greater than 100% using the mercury cleanup.

**MP245 Comparison of Residual Characteristics of Azoxystrobin, Difenconazole and Tebuconazole Sprayed at Different Times Before Harvest in Ginseng** S. Park, H. Noh, K. Lee, J. Lee, Chungbuk National University; K. Kwon, J. Oh, J. Do, National Institute of Food and Drug Safety Evaluation; M. Im, Korea Food & Drug Administration; K. Kyung, Chungbuk National University.

**MP246 Determination of Henry's law constant for the herbicide benzo-bicyclon conducted under California rice field conditions** K. Williams, University of California Davis; R. Tjeerdema, University of California – Davis / Environmental Toxicology. The rise in herbicide resistance observed in California rice field weeds, such as *Scirpus juncoides* and *Lindernia attenuata*, has generated increasing interest in herbicides with alternative modes of action from those currently in use. One such herbicide is benzbicyclon, a triketone herbicide, which inhibits hydroxyphenylpyruvate dioxygenase (HPPD) activity which subsequently halts plant photosynthesis through the destruction of chlorophyll and leads to plant bleaching and death. The mode of action of this HPPD-inhibitor herbicide has the potential to overcome the problem of weed persistence, however more information concerning the partitioning of benzbicyclon in the environment should be obtained prior to its application to California rice fields. In this study we will attempt to elucidate the partitioning of benzbicyclon between air and water through the determination of its Henry's law constant, representative of volatilization potential, considering California rice field conditions. A gas-stripping apparatus with mist trap incubated at two temperatures, 25°C and 37°C, will be used to conduct this experiment. These temperatures have been chosen such that the range in temperatures observed by California rice fields will be accounted for. We will employ a mass balance approach for the analysis of benzbicyclon partitioning, wherein possible sorption of benzbicyclon to the gas-stripping apparatus will also be considered in conjunction with its partitioning into the nitrogen flow and water. Extracted samples will be analyzed by GC-MS and examined using a level I fugacity model.

**MP247 Developing a Web-based System for Computing Pre-Harvest Residue Limits (PHRLs)** H. Chang, National Agricultural Products Quality Management Service / Consumer Information and Food Safety Division; H. Bae, National Agricultural Products Quality Management Service / Experiment Research Institute; Y. Son, I. Song, C. Lee, N. Choi, J. Chai, Y. Lee, National Agricultural Products Quality Management Service. This study describes the development of a web-based system that collects all data generated in the research conducted to set pre-harvest residue limits (PHRLs) for agricultural product safety control. These data, including concentrations of pesticide residues, limit of detection, limit of quantitation, recoveries, weather charts, and growth rates, are incorporated into a database, a regression analysis of the data is performed using statistical techniques, and the PHRL for an agricultural product is automatically computed. The development and establishment of this system increased the efficiency and improved the reliability of the research in this area by standardizing the data and maintaining its accuracy without temporal or spatial limitations. The system permits automatic computation of the PHRL and a quick review of the goodness of fit of the regression model. The National

Agricultural Products Quality Management Service sets the PHRL for the purpose of assuring agricultural product safety in the production stage. Furthermore, it evaluates the safety of agricultural products in the production stage according to the PHRL, investigates the amount of pesticide residues sprayed during the pre-harvest period, predicts the amount of pesticide residues at the time of harvest by computing the biological half-life and the decay constant, and prevents the distribution of unsuitable agricultural products that may exceed the MRL.

**MP248 Ecotoxicological tests to evaluate the acute toxicity and increase of biomass of earthworms acclimated in fresh and vermicomposted organic residue** L.B. Pigatin, IQSC-USP / IQSC; F. Benetti, A. dos Santos, L. Mendes, M. Kanashiro, R. Rachide, M. Landgraf, M. Rezende, IQSC-USP.

The final destination of agroindustrial organic residues is an eminent environmental problem in the world. The carbon applied through organic residues can lead to inadequate soil-atmosphere dynamics and favor mechanisms that negatively influence climate change. The organic material applied to the soil may find conditions that favor its decomposition, emitting a large amount of CO<sub>2</sub> into the atmosphere and, along with other gases, increasing the greenhouse effect. Vermicomposting is an ecologically alternative to the final disposition of organic residues. This work evaluates the acute toxicity and increase of biomass of earthworms *Eisenia fetida* acclimated in fresh and vermicomposted organic residue (filter cake). The earthworms were used as a bioindicator in the impact evaluation of application of the fresh and vermicomposted filter cake in the soil. The tests for evaluation of the acute toxicity were based on ISO 11268-1 and ISO 11268-2. Experiments were conducted in São Carlos city, SP, Brazil. The experiment factorial design was 2x3x5, 2 different treatments applied in 3 different doses and tests were conducted in quintuplicate using a native Oxisol. The treatments were: FFC (soil+fresh filter cake) and VFC (soil+filter cake vermicompost). Equivalent doses applied in the soil were: 0.5, 1.0 and 3.0% of the soil mass (500 g), and 0% for control. Humidity was maintained at 60% and 10 adult earthworms were added in each pot. To evaluate the acute toxicity and increase of biomass the earthworms were counted and weighed with 0, 7 and 14 days. The filter cake was obtained from the sugar and ethanol industries. The wet residues were subjected to the vermicomposting for during 6 months. The results demonstrated that the initial matrices (FFC and VFC) presented no acute toxicity for the earthworms. The earthworms presented an increase of biomass during the first 7 and 14 days. However for the fresh residue the increase of biomass was greater indicating that fresh filter cake provides more labile carbon as a source of nutrients for the earthworms. After 7 and 14 days treatment FFC showed 32.07% and 44.77% of biomass gain over the control. While after 7 and 14 days treatment VFC showed 14.64% and 23.96% of biomass gain over the control, both in its maximum dosage. The results showed that the treatments did not cause a harmful effect on the earthworm.

**MP249 Fate and effects of clothianidin in agricultural fields** C. de Perre, B. Hanson, W. Hanson, M. Lydy, Southern Illinois University. Neonicotinoids are systemic insecticides that have been under recent scrutiny due to their suspected toxicity to non-target species, including honey bees. The objectives of this project were to evaluate tillage use and treatment concentrations on the fate of clothianidin, a neonicotinoid insecticide, on fields with corn/soybean annual rotation. In order to better understand persistence and transport of neonicotinoids, soil, run-off sediments, infiltration water, and ground water samples were collected throughout two farming seasons. The planting event occurred in 2011 with corn seeds coated with clothianidin at two concentrations and the fate and transport of clothianidin was monitored for two years. The field was split in three parts where two underwent low-till processes (Aerway and Turbo till) and the third one underwent no vertical tillage. Clothianidin was detected in almost all soil samples, including the ones collected a year after planting, showing persistence of this compound in soil. Clothianidin concentrations in soil and water samples will be discussed in an attempt to correlate seed coating rate and tillage use to the fate and transport of this compound. In order to evaluate the effects of clothianidin at field concentrations, laboratory acute toxicity tests have been performed on several non-target species including the aquatic amphipod (*Hyalella azteca*), microcrustacean (*Daphnia magna*), midge (*Chironomus dilutus*), fathead minnow (*Pimephales promelas*), earthworm (*Eisenia fetida*), and duckweed (*Lemna minor*). The median lethal concentrations (LC50, concentrations that results in 50% organism mortality) and the median effective concentrations (EC50, concentrations that results in 50%

affected organisms) have been calculated for each species tested. Clothianidin was shown to have no significant effect on *Lemna minor* growth at concentrations up to 1000 µg/L. Acute LC50 values were in the same range for *Hyalella azteca* and *Chironomus dilutus*, 55 and 26 µg/L, respectively. The LC50 and EC50 values for each species will then be compared to soil and water concentrations of clothianidin measured in the field to assess the environmental risk of this insecticide.

**MP250 Fate of a transgenic insecticidal protein, a pyrethroid insecticide, and neonicotinoid insecticides within a maize agricultural ecosystem** S. Mueting, Southern Illinois University Carbondale / Department of Zoology, Southern Illinois University Carbondale / graduate student; M. Lydy, Southern Illinois University Carbondale / Fisheries and Illinois Aquaculture Center and Department of Zoology, Southern Illinois University-Carbondale / Fisheries and Illinois Aquaculture.

With the increasing prevalence of transgenic crops used in combination with other insecticides, the ultimate fate of these pesticides is critical to understanding potential effects to non-target species. Concentrations of Cry1Ab, a transgenic insecticidal Bt protein used in maize; tefluthrin, a pyrethroid insecticide; and thiamethoxam and clothianidin, neonicotinoid insecticides used as a seed coating were measured in soil, runoff water, groundwater, and soil pore water before, during, and after maize planting for two years. A 90 acre farm in Christian County, IL was divided into separate non-Bt and Bt fields with three different rates of tefluthrin applied in replicate strips in both fields. Samples were analyzed for Cry1Ab using enzyme linked immunosorbent assays; for tefluthrin, samples were analyzed using GC-ECD; and for neonicotinoids, samples were analyzed using HPLC-DAD and HPLC-UV. In 2010, 100 soil and 150 water samples were processed for all three insecticides; and in 2011, 140 soil and 125 water samples were processed. Cry1Ab proteins were found in soil at low concentrations throughout the growing season in soils collected from the Bt field. Only a few soil samples collected from the non-Bt field contained Cry1Ab. Runoff water samples from both fields frequently contained Cry1Ab and were at the highest concentrations of any water samples. Groundwater and soil pore water often did not have detectable concentrations of Cry1Ab. Tefluthrin was detected at the highest concentrations in soil samples, and was, in general, not found to be transported by water. Neonicotinoid seed coating compounds were detected in water and soil samples throughout the growing season with the highest concentrations found in soil pore water samples. Concentrations of all three pesticides were low or near the reporting limit before planting indicating that most of the chemicals and proteins have been metabolized or broken down in the environment before the next growing season. While Cry1Ab proteins were not found in environmental matrices at ecologically relevant concentrations, tefluthrin and neonicotinoids often were detected at or above documented LC50 literature values during the growing season.

**MP251 Influence of the presence of vermicompost in the toxicity of glyphosate on *Eisenia foetida* earthworms** F. Benetti, L. PIGATIN, L. Mendes, M. Kanashiro, A. dos Santos, R. Nunes, M. Landgraf, M. Rezende, IQSC-USP.

Earthworms are used as bioindicators for evaluation of soil contamination, because they ingest large amounts of soil, accumulating pollutants and recycling nutrients in this compartment. The objective of the study was to evaluate the influence of the presence of vermicompost (from filter cake and cattle manure) in the biomass and glyphosate toxicity to *Eisenia foetida* earthworms. The acute toxicity tests were performed according to ISO 11268-1 and 11268-2. All assays were performed with five replicates, each result is the arithmetic mean of these replicates. Fifteen pots were assembled: five control pots (500 g of soil – sandy silty fine to medium dark brown with organic matter), five pots with 500 g of soil and 10 mL of 10,000 mg kg<sup>-1</sup> of glyphosate solution (Glyphosate 480 A KB – Kelldrin®) and five pots of soil, 15 g of vermicompost and 10 mL of glyphosate solution. In each pot, the humidity was maintained at 60% and 10 adults earthworms were placed. For biomass and acute toxicity monitoring, earthworms were weight on days 0, 7 and 14. Compared to control, after seven days, in the experiments with no vermicompost, a 21% decrease on biomass and 4.2% decrease on mortality were observed. On the other hand, in the experiments with addition of vermicompost, a gain of 3.5% on biomass and a growth of 8.3% were observed. After fourteen days, compared to control, the same behavior was noticed: to experiments with no vermicompost, a loss over 8.3% of biomass and 8.2% of mortality were observed. In the experiments with addition of vermicompost, a gain of 24.8% on biomass and a growth of 6.2% were observed. It is worth to notice that the presence

of vermicompost can unavailable the glyphosate to the earthworms. The experiments showed that the concentration of 10,000 mg kg<sup>-1</sup> of glyphosate presents acute toxicity for the earthworms because interfere with their development since the beginning, which is demonstrated by the losses on biomass, but the presence of vermicompost helps to minimize this effect.

**MP252 Monitoring of Pesticide Residues in Environment-friendly Fruit and Fruiting Vegetable** J. Lee, H. Noh, k. Lee, S. Park, Chungbuk National University; S. Hong, Rural development administration; K. Kyung, Chungbuk National University. This study was carried out for the risk assessment of pesticide residues in environment-friendly fruit and fruiting vegetables collected from markets in Korea. A total of 555 (152 organic agricultural products, 202 pesticide-free agricultural products, 201 low-pesticide agricultural products) fruit and fruiting vegetable samples were collected twice from markets in May and August 2011 in Korea. The samples were selected from 25 types of the commodities that were readily available for retail sale. Pesticide residues in samples were analyzed by multiresidue method for 240 pesticides using GC-ECD/NPD and HPLC-DAD/FLD. The suspected-pesticides were confirmed with a GC-MSD. Four pesticides were detected from samples, representing a detection rate of 0.72%. EPN was detected from the organic pear at 0.2 mg/kg, representing that the organic pear for which organic synthetic agrochemicals and chemical fertilizers are not used violated Korean law because of pesticide detection. Also, bifenthrin, EPN, and chlorpyrifos were detected at levels less than half of its MRL from the low-pesticide agricultural products such as apple, Korean melon and peach, indicating that the pesticides detected from the low-pesticide agricultural products for which organic synthetic herbicides are not used and synthetic agrochemical/chemical fertilizers are used below half of standard amount met its certification criteria. No pesticide was detected from pesticide-free agricultural products. Result of risk assessment for the detected pesticide residues showed that the residue levels were below their MRLs. Also estimated daily intakes (EDIs) of the pesticides detected from fruit and fruiting vegetables were less than 4.2% of their acceptable daily intakes (ADIs), indicating that residue levels of the detected pesticides were safe for consumption.

**MP253 Partitioning of Imazosulfuron under California Rice Field Conditions** C. Rering, University of California, Davis / Agricultural and Environmental Chemistry Graduate Group; R. Tjeerdema, University of California – Davis / Department of Environmental Toxicology. Imazosulfuron (trade names: Brazzos, League) is a sulfonylurea herbicide of interest to California rice farmers due to its rice selectivity and high effectiveness at a low application rate (75-95 g ha<sup>-1</sup>). Partitioning to air and soil can be determined by experimentally derived Henry's law constants ( $K_H$ ) and organic carbon-normalized soil-water distribution coefficients ( $K_{oc}$ ), respectively. Samples from both air-water and soil-water partitioning experiments were analyzed by LC/MS/MS. Imazosulfuron's  $K_H$  was investigated by the gas stripping method at 25 and 35°C and by estimation based on known chemical properties. Imazosulfuron did not significantly volatilize from the aqueous phase and did not exhibit significant temperature dependence. This correlates well with the calculated Henry's law constant of 6.03 x 10<sup>-7</sup> m<sup>3</sup> Pa mol<sup>-1</sup>. Soil-water partitioning will be determined by the batch equilibrium method for soils collected in California's Central Valley, at representative temperatures.

**MP254 Pre-Harvest Residual Characteristics of Ethaboxam and Chlorantraniliprole in Cucumber** S. Yun, E. Park, National Agricultural Products Quality Management Service; Y. Kim, National Agricultural Products Quality Management Service; S. Shim, C. Jin, J. Ryu, H. Choi, National Agricultural Products Quality Management Service; H. Noh, K. Kyung, Chungbuk National University. This study was carried out to investigate the residue patterns and estimate the dietary intakes of the commonly used fungicide ethaboxam and insecticide chlorantraniliprole in cucumber under greenhouse conditions. The pesticides were sprayed two times at a seven day intervals onto the cucumber until 10 days before harvest and then sampling was done at 0, 1, 2, 3, 4, 6, 7, 8 and 10 days after the last spraying. The analytical samples were prepared by QuEChERS method and the pesticides residues were determined with an LC-MS/MS. Limits of detection of the pesticides were 0.001 mg/kg for both ethaboxam and chlorantraniliprole and mean recoveries of ethaboxam and chlorantraniliprole were from 91.7 to 100.8% and from 81.9 to 90.8%, respectively. Biological half-lives of ethaboxam were 2.8 days at one time spraying and 2.5 days at two time

spraying, while those of chlorantraniliprole were 3.1 days at one time spraying and 3.8 days at two time spraying. The estimated daily intakes (EDIs) of the pesticides in the crop harvested at 10 days after spraying was less than 0.2% of their acceptable daily intakes (ADIs), representing that risk of the pesticide residues in cucumber would be very low.

**MP255 Residual Characteristics and Processing Factors of Difenoconazole in Fresh Ginseng and Processed Ginseng Products** H. Noh, k. Lee, J. Lee, S. Park, Chungbuk National University; K. Kwon, J. Oh, J. Do, National Institute of Food and Drug Safety Evaluation; M. Im, Korea Food & Drug Administration; K. Kyung, Chungbuk National University. This study was carried out to survey the residual characteristics and calculate its processing factors of difenoconazole in ginseng and its processed products, such as dried ginseng, red ginseng and their water and alcohol extracts. The pesticide was sprayed onto the ginseng according to its pre-harvest intervals in 2009 (four-year-old ginseng) and 2010 (five-year-old ginseng). Harvested ginseng was processed to dried ginseng, red ginseng and their extracts according to the commercially well-qualified conventional methods provided by the Korea Ginseng Corporation. Limit of detection (LOD) and limit of quantitation (LOQ) of difenoconazole in fresh ginseng were 0.001 and 0.003 mg/kg, respectively. In case of processed ginseng products, their levels were 0.002 and 0.007 mg/kg, respectively. Concentration of difenoconazole in both fresh ginseng and its processed products increased with the experimental period. Processing factors, calculated as a ratio of difenoconazole concentration in processed products to fresh ginseng were found to be 1.71 to 2.17 and 1.62 to 2.03 in case of dried and red ginseng, respectively, while those for their extracts ranged from 1.76 to 2.98. In case of five-years-old dried ginseng and red ginseng as well as their extracts, the ranges of processing factor of difenoconazole were found to be 2.9 to 3.1, 1.9 to 2.2 and 2.4 to 4.7, respectively.

**MP256 Residual Characteristics and Risk Assessment of Chlorantraniliprole, Teflubenzuron and Trifloxystrobin in Shallot** K. Kyung, H. Noh, k. Lee, J. Lee, S. Park, Chungbuk National University; C. Jin, S. Yun, National Agricultural Products Quality Management Service. This study was carried out to survey the residual patterns of chlorantraniliprole, teflubenzuron and trifloxystrobin; calculate their biological half-lives and evaluate the risk of these pesticides in shallot. The pesticides were sprayed once or twice at a seven day intervals before harvest and samples were taken sequentially from the day of spraying (0 day) to harvest (14 days). HPLC equipped with diode array detector (DAD) was used for the analysis of pesticide residues in plants. The method quantitation limits (MQLs) of all the three pesticides were 0.03 mg/kg and recoveries of chlorantraniliprole, teflubenzuron and trifloxystrobin in shallot ranged from 85.85 to 89.66, 93.91 to 96.67 and 94.17 to 101.23 mg/kg, respectively. The initial concentration of three pesticides exceeded their maximum residue limits (MRLs) at the both treatments of spraying, but concentration of chlorantraniliprole did not exceed its MRL in samples taken two days after treatment. Teflubenzuron did not exceed its MRL in samples collected two days after treatment in case of spraying once and also in samples collected four days after treatment in case of spraying twice. Trifloxystrobin levels stayed within its MRLs for the crop collected after 6 and 10 days before harvest for spraying once and spraying twice, respectively. The biological half-lives of chlorantraniliprole in cases of spraying once and twice were 3.3 and 3.7 days, respectively, and the half-lives of teflubenzuron equaled 3.6 days in both treatments. In case of trifloxystrobin, the half-lives were 4.4 days for spraying once and 5.5 days for spraying twice. Risk assessment for the pesticides resulted in chlorantraniliprole levels being less than 3% of their acceptable daily intakes (ADIs) at 0 and 14 days after last treatment. Teflubenzuron and trifloxystrobin were less than 27% of their ADIs at 14 days after last treatment.

**MP257 Soil bacteria that utilize fipronil as the only carbon source** L.T. Cappellini, J. Alberice, E. Pozzi, Sao Paulo University; D. Cordeiro, São Paulo University; L.G. Diniz, J.P. Lima, E. Carrilho, E.M. Vieira, Sao Paulo University. Due the great interest about knowing the soil microbiology, mainly because of intensive use of pesticides in agriculture, this work aims to determine bacteria of the soil capable to use the fipronil as unique carbon source. This compost is applied in sugar cane plantations, after the planting and during the planting; this procedure aims to form a barrier against insects. The soil used was a yellow red oxisol, collected in area under cultivation of sugar cane. In a sample of 10 g of soil was added 90 ml of sterile purified water left under stirring for 1 hour at 25 °C. After this period it



was collected an aliquot of 9 mL of this solution, which was transferred to a medium containing water and fipronil (1.66 mg L<sup>-1</sup>) and placed in a greenhouse at 40 °C without stirring. DNA was extracted from this sample using the method of Griffiths (2001) with modifications, then we make an electrophoresis on 0.8% agarose to confirm the efficiency of the extraction of DNA by this procedure. For the amplification of the extracted material it was applied PCR using a primer 27F/1100R according to the conditions proposed by Lane (1991) for programing the thermal cycler; and made again electrophoresis in 1.4% agarose gel to confirm the efficiency of amplification of DNA. After this phase the PCR product was cloned according to manufacturer's specifications Promega®. The cloned material was sent for sequencing. The nucleotide sequencing was done in sequencer ABI 377 DNA Sequencer (Perkin Elmer – USA). The sequences obtained were added and aligned using DNASTAR-Package Software (Lasergene Sequence Analysis) and grouped into operational taxonomic units (OTUs). After alignment and investigation about the presence of chimeras, the nucleotide sequences were compared to sequences of the electronic database-NCBI database for identity phylogenetic approach. The construction of the phylogenetic tree was done using the program Molecular Evolutionary Genetics Analysis (MEGA) version 5.05, and estimated by evolutionary distances Neighbor Joining method. According to the technique employed, the 96 clones sent for sequencing, 38.54% of agencies affiliated with 20.83% and 15.63% *sp* *Flavisolibacter* affiliated with *Burkholderia sp* the other 25% of clones sequenced are bacteria characterized not cultured in the laboratory yet.

**MP258 Determination of Glyphosate and AMPA by HPLC-UV and GC-MS** F. Benetti, IQSC-USP; B.M. da Silva, UNICAMP; M. Landgraf, M. Rezende, IQSC-USP. Glyphosate (GLY) is a herbicide with a great agronomic efficiency. Aminomethylphosphonic acid (AMPA) is its main metabolite. The objective of this work was to develop analytical methodology for determination of both. Using HPLC-UV, GLY is determined, and by GC-MS both could be determined. Environmental samples were determined throughout the suggested methodology. The determinations were performed on model LC-10AVP HPLC, with UV-Vis detector SPD-10VP, Shimadzu; mobile phase buffer (KH<sub>2</sub>PO<sub>4</sub>/H<sub>3</sub>PO<sub>4</sub> (85%) until pH 2), flow of 2,3 mL min<sup>-1</sup> with isocratic system; column, anion exchange, Partisil 10 SAX (4.6 x 250 mm); temperature, 25 °C e wavelength of 195 nm. For GC-MS, the equipment used was a GC-MS Shimadzu GC 2010 with auto sampler and the conditions were: oven temperature, 80°C; injector temperature, 280°C, interface temperature, 260°C; ions source temperature, 200°C; total flow, 15.1 mL min<sup>-1</sup>; column flow of 2.02 mL min<sup>-1</sup>; linear velocity, 57.3 cm s<sup>-1</sup>; splitless injection mode and 22.4 kPa pressure. Ions monitored were m/z 246 and 302 for AMPA and 238, 260 and 411 for GLY. For GC-MS, the method proved to be linear with correlation (R) and determination (R<sup>2</sup>) coefficients of 0.9963 and 0.9925, respectively. The limit of detection (LD) and of quantification (LQ) were 0.67 µg L<sup>-1</sup> and 2.02 µg L<sup>-1</sup>, respectively. The method proposed by HPLC-UV presented linear, with R and R<sup>2</sup> significant concentration within the range from 50 to 500 µg L<sup>-1</sup> of 0.9983 and 0.9966, respectively. For HPLC-UV, the values of LD and LQ, were 9.93 and 30.1 µg L<sup>-1</sup>, respectively. The mobile phase is an aqueous solution, ie, there is no risk to the environment. Another favorable aspect was a development of an analytical methodology without clean-up step, and no need of derivatization reactions. The methodology developed for GLY determination by HPLC-UV is considered sensible and rapid, and the methodology developed throughout GC-MS is able to determining GLY and AMPA, but spends more time for analysis. Both meet the current Brazilian legislations (CONAMA 357 and MS 2914), but the choice of the method is up to the laboratory, according to the needs.

**MP260 Early Life Stage Toxicity of the Intense Sweetener Sucralose to the Fathead Minnow** D.B. Huggett, University of North Texas / Department of Biological Sciences, University of North Texas / Institute of Applied Sciences. Emerging contaminants, such as pharmaceuticals, personal care products and food additives, are receiving considerable attention with respect to their environmental fate and toxicological properties. These contaminants are in constant commercial use and are thus being continually introduced into the environment via wastewater treatment plants (WWTP). This constant exposure scenario suggests that comprehensive environmental fate and ecotoxicological studies are needed to evaluate the environmental risks of emerging contaminants. Sucralose (1,6-dichloro-1,6-dideoxy-β-D-fructo-furanosyl 4-chloro-4-deoxy-α-D-galactopyranoside), sucralose is not readily degraded within the wastewater treatment process and has been

detected in municipal effluents and surface waters. A battery of acute (rainbow trout, bluegill sunfish, *Daphnia magna*, green algae) and chronic (mysid shrimp, *Daphnia magna*, *Lemna gibba*) ecotoxicological studies have been conducted, however no chronic fish data are available. To address this data gap, an OECD 210 Fish Early Life Stage Fish Study was conducted with the fathead minnow at concentrations up to 100 mg/L sucralose. No significant responses were observed in the any of the parameters monitored (survival, growth, etc). A wide margin of safety exists when these ecotoxicological data are compared to predicted or measured aqueous sucralose concentrations, indicating that sucralose presents a negligible risk to the environment.

**MP261 Environmental considerations with Detergent Formulations in the Household Sector** H. Kola, F. Pala, Battelle. Due to the environmental concern with some detergent ingredients, Battelle launched an annual analytical Detergent Program in 1987 to study the major new developments undertaken by the detergent industry. Initially the ban of phosphates in household laundry detergents and the need for a new builder system were the major interests of the companies participating in this multi-client program. Most Western European nations and the US have already banned the use of phosphates in laundry detergents; currently they are considering a similar ban for dishwasher detergents. Another formulation change that has had a positive environmental impact was the introduction of percarbonates in the bleaching system of laundry powders and restriction of perborates. Later on, the search for more biodegradable surfactants allowed raw material suppliers to take advantage of the opportunity to provide new ingredients such as sulfonated methyl esters, narrow range ethoxylates, cationic and amphoteric surfactants, glucose-derived alkyl polyglycosides, short-chain alpha-olefin sulfonates, ethoxylated methyl esters, short chain linear alcohol ethoxylates, etc. As product development and innovation continues to be a key driver in the laundry detergent business, the detergent manufacturers, the suppliers of ingredients, and the environmental agencies find it essential to follow these developments. Consequently the number of analyses to be carried out on commercial products has multiplied. This paper gives an overview of the most important changes in the household detergent sector that have had a positive environmental impact on the European and the US economies. Examples of commercial laundry and autodish detergents are discussed in detail. In addition it describes the tool, i.e. the analytical program, which made it possible to track these developments over the last 20 years.

**MP262 How a New Builder in Automatic Dishwashing Detergent Became Ready Biodegradable in the U.S.: Widespread Microbial Adaptation in the Field** N. Itrich, Procter & Gamble Company / Environmental Stewardship Organization, The Procter & Gamble Company / Environmental Safety Department; K. van Ginkel, AkzoNobel Technology and Engineering; E. Schaefer, Wildlife International; K. Casteel, J. Menzies, Procter and Gamble Company; M. Mathews, Wildlife International; E. Bisinger, J. Lepage, AkzoNobel Functional Chemicals; T. Federle, Procter and Gamble Company. L-GLDA (Glutamatediacetate) is a chelant that was introduced as a phosphate replacement in a North America (NA) automatic dishwashing (ADW) detergent gel in early 2010. Prior to ADW use, L-GLDA failed multiple biodegradation screening tests (OECD301B) in the NA region and was not considered ready biodegradable in this geography. However, results from a more realistic continuous activated sludge treatment test (OECD 303A) predicted that a microbial population pre-exposed to L-GLDA would extensively degrade it after a lag period. In this same time frame, activated sludge inoculum from a single EU plant did degrade L-GLDA and produced a bacterium that could utilize it as a sole nitrogen, carbon and energy source. The market introduction of this new ADW and the subsequent widespread distribution of L-GLDA in wastewater offered a unique opportunity to monitor for potential microbial adaptation in the field. After launch of the ADW, a series of OECD 301B tests were conducted using inoculum collected from multiple WWTPs in two NA regions. Within several months, WWTP inoculums that had previously produced negative results, measuring 9-21% CO<sub>2</sub> production, demonstrated that L-GLDA was "ready biodegradable" (65-100% CO<sub>2</sub>). Inoculum from additional WWTPs also showed positive ready test results and a decrease in the measured lag period (< 10% CO<sub>2</sub>) was observed as distribution of the ADW grew, indicating an increase in the field population of competent degraders. This is the first systematic study showing how a new chemical, that consistently failed ready biodegradation tests in the U.S. became ready biodegradable across a wide geographical region following its introduction

in a commercial product. The ready test is required under most regulatory schemes for the registration of a new chemical and to demonstrate a lack of persistence. However the use of pre-exposed inoculum is strictly prohibited in some regions. This work demonstrates that when pre-exposure occurs under realistic conditions, laboratory tests can accurately predict ultimate real world behavior indicating the importance of considering potential adaptation in regulatory assessments.

**MP263 In vitro comparative pharmaceutical metabolism with fathead minnow (*Pimephales promelas*) and rainbow trout (*Oncorhynchus mykiss*) liver S9** K.A Connors, Baylor University / Institute of Biomedical Studies, Baylor University / Department of Environmental Science; B. Du, Baylor University / The Institute of Ecological, Earth, Environmental Sciences, Baylor University / Department of Chemistry and Biochemistry; Department of Environmental Science, Baylor, Baylor University / The Institute of Ecological, Earth, Environmental Sciences, Baylor; P.N. Fitzsimmons, U.S. Environmental Protection Agency / ORD, NHEERL, Mid-Continent Ecology Division; C.K. Chambliss, Baylor University; J.W. Nichols, U.S. Environmental Protection Agency / ORD, NHEERL, Mid-Continent Ecology Division; B.W. Brooks, Baylor University / Environmental Science and Biomedical Studies, Baylor University / Department of Environmental Science. The occurrence of pharmaceuticals in the environment represents an area of emerging concern because chemical and biological properties of therapeutics present unique challenges to ecological risk assessment. Unlike other industrial chemicals, existing pharmaceutical safety data and pharmacology information may be leveraged through biological "read-across" approaches to aid environmental assessments. However, few approaches and robust empirical datasets exist, particularly for comparative pharmacokinetic applications. We recently performed rainbow trout S9 metabolism studies for a variety of common therapeutics primarily metabolized by specific CYPs in mammals. These results suggest that substrate specific metabolism patterns and enzyme generalizations between mammals and fish models may be difficult to make. Specifically, pharmaceuticals that are either substrates for CYP2D6 or general CYP substrates in mammals exhibited low rates of clearance in rainbow trout liver S9. Additionally, no significant pharmaceutical metabolism was observed in CYP3A4 and CYP2C9 substrates. Given the high degree of metabolic enzyme conservation among fish, cross species extrapolations may still be possible. Finding a way to extrapolate metabolism data among fish species would be ideal, however the limits of this approach still remains relatively unknown. As a step towards this goal, we employed a comparative metabolism approach to compare the metabolic capabilities between rainbow trout and fathead minnow liver S9. Results from this effort will help inform the advantages and limitations of cross-species extrapolations of comparative metabolism and pharmacokinetics during fish toxicology studies and environmental risk assessments of pharmaceuticals.

**MP264 In vitro metabolism of deltamethrin by hepatic S9 from adult zebrafish (*Danio rerio*)** K.C Pangallo, Colgate University / Chemistry; T.S. Kung, Rutgers, the State University of New Jersey; K.R. Cooper, L.A. White, Rutgers, the State University of New Jersey / Department of Biochemistry and Microbiology. Pyrethroid pesticides are commercially available products that are present in thousands of consumer products. Pyrethroids are less acutely toxic than many other pesticides, such as organophosphates. The extensive use and chronic exposure to children by pyrethroid pesticides has sparked investigations into the toxic effects resulting from these exposures. In the developing zebrafish (*Danio rerio*) low doses of the pyrethroid pesticide deltamethrin (0.5 µg/L) resulted in behavioral changes and alterations in the expression of genes important to proper neuronal development. The nominal deltamethrin dosages and mechanism of action responsible for these alterations are being examined. However, in order to apply results from this model system to other vertebrates, the concentration of deltamethrin within the organism must be considered. One factor that can greatly affect the concentration of a compound within tissues is metabolism. Here we present results from incubations of 0.0010 M deltamethrin with hepatic S9 fractions isolated from zebrafish tissue. The incubations were conducted at 37 °C for 1 hour in phosphate buffer (pH 7.2) with and without NADPH. The deltamethrin metabolism was determined by HPLC. The concentration of deltamethrin was reduced by approximately 50% with and without NADPH (possibly due to residual NADPH in the S9 fractions). The concentration of deltamethrin in control incubations (deltamethrin in buffer) did not result in any appreciable loss.

Zebrafish hepatic S9 can metabolize deltamethrin and further characterization of the metabolites formed and specific tissue metabolic rates will allow for comparison to higher vertebrates and common mechanisms of action. (NIEHS ES07148, EOHHS ES05022)

**MP265 Source apportionment of micro-plastic originating from personal care products** T. Gouin, Unilever / Safety and Environmental Assurance Centre. There has been an increasing trend to use polyethylene micro-plastic material in personal care products as an exfoliating agent. Recent activity, however, has linked the presence of plastics in the environment to the potential for increased exposure of organisms to certain pollutants through ingestion of contaminated plastic. Given that emissions of micro-plastic to the global marine environment include both primary sources (as derived from hand and facial cleansers, cosmetic preparations, airblast cleaning media, and production waste from plastic processing plants) and secondary sources (derived from fragmentation of macro-plastic as a result of photodegradation and abrasion due to wave action), there is a need to better characterise the relative importance of sources of micro-plastic material to the environment. Using the consumer market products database, Euromonitor International, we have calculated the use of polyethylene micro-plastic used in personal care and have linked this information with estimates of removal via waste water treatment to quantify the release of micro-plastic originating from personal care products. In the U.S. the per capita consumption of micro-plastic used in liquid soaps is estimated to be 2.4 mg/d. Based on knowledge that 25% of the U.S. population is connected to septic systems, with a significant fraction connected to waste water treatment systems utilising trickling/sand filtration processes, it is demonstrated that a relatively small volume of micro-plastic used in personal care is emitted to the aquatic environment. It is further demonstrated that micro-plastic originating from secondary sources are the dominant exposure route. It is anticipated that an improved understanding of sources will strengthen our ability to appropriately assess the risks of micro-plastic in the environment.

**MP266 The use of LC-MS/MS and GC-MS methods for the quantification and characterization of anionic surfactants in detergent formulations** J. Seyfert, Battelle; M.J. Benotti, Battelle / Analytical and Environmental Chemistry; C. Usher, W. Li, Battelle. Anionic surfactants are the most abundant surfactants used in both liquid and powder laundry detergents commercialized worldwide. Over the past decades, an increasing variety of anionic surfactants, such linear alkylbenzene sulfonate, alkyl sulfates and ethoxy sulfates, fatty acid methyl ester sulfonate, secondary alkane sulfonate, alpha-olefin sulfonate, and others were introduced to the market and massively used in synthetic detergent formulation. The development of analytical methods to ensure the accurate identification, quantification, and speciation of anionic surfactants in detergent is crucial not only in the understanding of detergent market trends and cleaning properties, but also in modeling the environmental fate of this class of surfactants once the cleaning cycle is completed and detergents are discharged to wastewater treatment and ultimately to the environment. Modeling the environmental fate of surfactants requires data on the concentration of each surfactant type, the C-chain length distribution of the surfactant hydrophobic tale, whether the C-chain is linear or branched, the ethoxilation number (in the case of alcohol ethoxy sulfates), and the isomeric distribution (in the case of alkylbenzene sulfonate and secondary alkane sulfonate). This paper presents a systematic approach to the identification, characterization, and quantification of anionic surfactants in detergent formulation by using an array of LC-MS/MS and GC-MS analytical methods that require minimum sample preparation and allows the characterization of a large number of detergents.

**MP267 Tissue and Intra-Brain Distribution of Perfluoroalkyl Carboxylates and Sulfonates, and Select Precursors and Isomers in East Greenland Polar Bears** A.K. Greaves, Carleton University / Department of Chemistry; R.J. Letcher, Carleton University / Department of Chemistry, Environment Canada / Science and Technology Branch, Ecotoxicology and Wildlife Health Division, Ecotoxicology and Wildlife Health Division, Science and Technology Branch / Environment Canada; C. Sonne, R. Dietz, Aarhus University / Department of Biological Sciences; E.W. Born, Aarhus University / Greenland Institute of Natural Resources. Per- and poly-fluoroalkyl substances (PFASs) are widespread environmental contaminants, and bioaccumulative perfluoroalkyl acids (PFAAs) continue to be reported in wildlife worldwide. Although several PFAAs (such as perfluorooctane sulfonate (PFOS)) have been shown to bind to a wide variety of proteins



and appear to accumulate preferentially in the liver, the specific mechanisms and pathways of distribution in the body remains understudied. Recent mammalian tissue distribution studies have shed light on the importance of understanding the distribution mechanisms of these compounds in the body, but no such study to date has examined their distribution, particularly of perfluoroalkyl carboxylates (PFCAs) and perfluoroalkyl sulfonates (PF-SAs), in an Arctic mammal. In the present study, we compared the patterns and concentrations of PFCAs ( $C_6 - C_{15}$ ), PFSA's ( $C_4, C_6, C_8, C_{10}$ ), and some of their precursors (e.g. perfluorooctane sulfonamides), as well as linear and branched isomers of PFOS, in the liver, whole blood, muscle, adipose, and brain (8 compartments) of 20 East Greenland polar bears (*Ursus maritimus*) collected in 2006. The liver contained by far the highest concentrations of PFASs, followed by whole blood > brain (inner > outer compartments) > muscle  $\approx$  adipose. However, large differences in the accumulation profiles of the PFCAs could be seen between all tissues, e.g. longer-chain PFCAs accumulated in the brain. Specifically in the brain, compartmental differences were observed on a wet weight basis, but these differences became statistically insignificant ( $p > 0.05$ ) on a lipid weight basis. In fact, the distribution of the longer-chain PFCAs ( $C_{10} - C_{15}$ ) were statistically correlated with extractable lipid content in the brain, such that brain regions with higher lipid contents also possessed higher concentrations of the  $C_{10} - C_{15}$  PFCAs. This lipid dependence has not been previously reported for any PFAS in any other tissue, suggesting protein interactions with PFCAs at the blood-brain barrier. Finally, linear as well as mono-branched PFOS was found in the liver and blood, whereas only linear PFOS was detectable in all other tissues. Overall, there were tissue-specific and/or brain compartment-specific levels and patterns of PFCAs and PFSA's and PFOS isomers in these polar bears, which sheds new light on the processes governing the pharmacokinetics and potentially the pharmacodynamics of these PFASs in mammals and perhaps other wildlife.

**MP268 Triclocarban Toxicity: A multi-Generational and Demographic Assessment Using *Daphnia magna*** B. Andrews, University of Guelph; P. Sibley, University of Guelph / School of Environmental Sciences, University of Guelph / Department of Environmental Biology. Triclocarban (TCC), an antimicrobial constituent in numerous personal care products, is commonly detected in North American surface waters at sub- $\mu\text{g/L}$  concentrations. The acute toxicity of TCC to aquatic organisms has been relatively well studied but information on toxicity in long-term exposures to environmentally relevant concentrations is lacking. In this study, we assessed the toxicity of TCC in a multi-generational test with *Daphnia magna* in laboratory exposures. 24-hr old *D. magna* neonates were exposed to TCC (nominal concentrations: 0.1-4  $\mu\text{g/L}$ ) over 3 generations (3 successive 21-d life cycle tests). Neonates randomly collected from 5 replicate beakers within each treatment on day 21 were used to seed each generation. Control survival exceeded 90% in all treatments across all generations; survival in the TCC treatments ranged from 50-100% and did not exhibit a concentration-response relationship. No significant effect on time to first brood was observed in any generation. Total offspring produced over 21 d and fecundity (average offspring/adult) decreased significantly with increasing TCC concentration in the second and third generations but not the first, with the effect more pronounced in the third generation. Consequently, the intrinsic rate of increase ( $r$ ) and predicted population size declined significantly with increasing concentration in the second and third generations. Measured concentrations indicated that actual exposures were approximately 50% of the nominal targets indicating that the observed effects occurred at TCC concentrations comparable to those found in surface waters. These data indicate potential risks to aquatic organisms in situations of long-term exposures to TCC.

**MP269 Acute and Chronic Toxicity of Ag Nanoparticles to *Daphnia magna* through Aquatic and Ingestion Routes of Exposure** A.J. Gibson, Western Washington University / Environmental Science; R.M. Sofield, Western Washington University / Huxley College of Environment. Silver nanoparticles (AgNP) from consumer products may enter aquatic systems through a number of different routes and because of the antimicrobial properties of silver, the assortment of consumer products containing AgNP has increased over the years. These products include treated shirts and socks, hand sanitizers, children's toys, and tooth brushes. This study aims to determine both acute and chronic toxicity to *Daphnia magna* using  $\text{AgNO}_3$  and Silver Citrate nanoparticles (AgNP-Cit) with and without humic acids. A modified version of the ASTM E729-07 method was used for acute toxicity testing, with no chloride added to the synthetic hard water solution to avoid

precipitating the silver. The chronic study will use *Selenastrum* that has been pre-exposed to  $\text{AgNO}_3$  and AgNP-Cit as a food source for the *D. magna*. Characterization of the nanoparticles will include digital light scattering (DLS) for size confirmation, ultraviolet-visible spectroscopy (UV-Vis) for the determination of sedimentation rate, and ion selective electrodes (ISE) for the measurement of the percentage of silver ions. The acute  $\text{EC}_{50}$  is 0.04 $\mu\text{g/L}$  for AgNP-Cit and 0.0002 $\mu\text{g/L}$  for  $\text{AgNO}_3$ . Results of this study will help define the contribution of the ionic Ag to the nanoparticle toxicity for both acute and chronic exposures, clarify the role of humic acids in toxicity modification, and contribute understanding to the role of trophic transfer in silver nanoparticle toxicity.

**MP270 Behavior of Silver Nanoparticles and interaction with Dissolved Organic Matter and temperature in *Caenorhabditis elegans*** X. Yang, / PHD student; A.R. Badireddy, C. Jiang, Duke University; H. Hsu-Kim, M. Wiesner, D.E. Hinton, Duke University / Civil and Environmental Engineering; J.N. Meyer, Duke University / Nicholas School of the Environment. The rapidly increasing manufacture of silver nanoparticles (Ag NPs) has raised both ecological and human health concerns. A key question is how to extrapolate laboratory results obtained under carefully controlled conditions to complex environmental media. In the present study, the nematode *Caenorhabditis elegans* was exposed to a silver nanoparticle (Ag NP) suspension with or without pre-incubation with natural organic matter (NOM).  $\text{AgNO}_3$  was used to isolate the effect of silver ion.  $\text{LC}_{80}$ 's were used for  $\text{AgNO}_3$  (0.15 ppm), non-coated Ag NPs (0.15 ppm), PVP-coated Ag NPs (8 nm) (0.5 ppm) and PVP-coated Ag NPs (38 nm) (1.5 ppm). We explored the impact of NOM (Suwannee River and Pony Lake fulvic acids) on Ag NP toxicity, and found that Pony Lake fulvic acid rescued toxicity more effectively. To explore the NOM effect in more depth, we are carrying out a series of toxicokinetic examinations using darkfield hyperspectral microscopy, transmission electron microscopy and physicochemical measurements (DLS and ICP-MS). In addition, we studied the impact of temperature (15, 20, and 25° C) in Ag NPs, finding increased toxicity at higher temperatures for all tested nanoparticles.

**MP271 Determination of Ion-Release Kinetics from Nanosilver in Aqueous Media of Varying Ionic Strength** A. Harmon, US Army Engineer Research and Development Center / Environmental Laboratory; A.J. Kennedy, US Army Engineer Research and Development Center, CEERD-EPR; A.R. Poda, US Army Engineer Research and Development Center; A.J. Bednar, US Army Engineer Research and Development Center / Environmental Laboratory; J.A. Steevens, U.S. Army Engineer Research & Development Center / Waterways Experiment Station. Recently there has been concern over the increase production of nanomaterials and their potential effects in aquatic environments. In the present study, the silver ion-release kinetics of 20, 50, and 80nm silver nanoparticles (AgNPs) in environmentally relevant waters was examined using concentrations of 10% and 50% moderately hard reconstituted water (MHRW) and the associated impact on an aquatic organism. Acute toxicity of the AgNPs suspensions was assessed using *Daphnia magna* at 0 and 7-days post interaction with media. Dissolution is a vital environmental transformation that affects the form and concentration of AgNPs in the environment. Determining the rate at which AgNPs dissolve will be the key factor in determining the fate of AgNPs in the environment. This study is aimed to answer fundamental questions that remain to be answered. Diluted suspensions of nanoparticles were placed on a multi-tube vortexer and orbital shaker for 0,1,2,3 and 7 days. Upon removal from the orbital shaker and vortexer total silver measurements and dissolved silver measurements (ultra centrifugation, followed by ICP-MS analysis) were taken. Dynamic Light Scattering (DLS) and Field Flow Fractionation (FFF) were used to determine particle size at each time point by measuring the relative change in particle size over time. A significant increase in particle size over time was observed in 100 and 50% MHRW but only a slight increase in 10% MHRW. Results showed a consistent increase in dissolved silver and decrease of total silver from day 0 to 7. This suggests that initially the concentration of dissolved silver will increase once in environmentally relevant media, but the longer term equilibrium between nanoparticle and dissolved fractions and associated biological effects remains poorly understood and further research is needed. There was an increase in toxicity with greater interaction time with media post 7 days. It was also shown that ions drive acute silver toxicity and lower ionic strength media allows greater particle stability and ion release thus greater toxicity.



**MP272 Environmental fate of silver nanoparticles in littoral mesocosms** L. Furtado, Trent University / Environmental and Life Sciences; E. Hoque, Trent University / Environmental & Resource Studies; H. Hintelmann, Trent University / Chemistry; C.D. Metcalfe, Trent University / Environmental & Resource Studies, Trent University / Department of Environmental & Resource Studies. Silver nanoparticles (AgNPs) are widely used in textiles and in medical applications for their antimicrobial properties. There is concern that AgNPs will be released from these products and enter aquatic ecosystems via municipal and industrial wastewaters, and these discharges will put aquatic organisms at risk. In order to accurately define these risks, the environmental fate of AgNPs once they enter a freshwater ecosystem must be understood. AgNPs discharged into water can remain as isolated particles, aggregate with other particles (homo-aggregation) or with suspended particulates (hetero-aggregation) and/or release dissolved silver (e.g. Ag<sup>+</sup>). These chemical transformations will dictate the environmental fate and resulting ecological risks associated with release of these contaminants of emerging concern. However, there are currently few analytical methods that can be used to characterize both the size and the form of AgNPs in aquatic matrices. In laboratory experiments, we refined "cloud point extraction" as a technique to pre-concentrate AgNPs suspended in water. Using this method, we extracted AgNPs capped with different agents at efficiencies >75%, while not extracting dissolved silver present in the water. Studies are currently underway to determine whether extracted AgNPs retain their size characteristics. This cloud point extraction technique as well as ultra-filtration are being used to characterize the transformations and distribution of AgNPs in mesocosms deployed in a natural lake environment as part of the Lake Ecosystem Nanosilver (LENS) project in the Experimental Lakes Area (ELA) of northwestern Ontario. Twelve littoral mesocosms (2 m diameters x 2 m depth) were installed in Lake 239 at ELA to evaluate the fate of polyvinylpyrrolidone (PVP) versus citrate capped AgNPs. After sample processing using both ultrafiltration and cloud point extraction, water samples are being analyzed by ICP-MS to determine the concentrations of silver in both dissolved form and nanoform. The results from this study will be presented and related to assessments of the risks associated with the release of AgNPs into the aquatic environment.

**MP273 The Influence of Capping Agent, Ion Concentration, and Humic Acids on the Toxicity of Silver Engineered Nanoparticles** R. Combs; C. Murdock, Western Washington University / Huxley College of the Environment; R.M. Sofield, Western Washington University / Huxley College of Environment. Engineered silver nanoparticles (AgENPs) are an emerging technology with rapidly increasing uses on the market today. Most often AgENPs are used in consumer products as a bactericide, however very little is known about the mode of action behind this toxicity. Furthermore, even less is known on the environmental implications once AgENPs enter the environment. Capping agents can further influence the fate and mobility of AgENPs in the aquatic environment, although little is known on the direct effects on toxicity of these agents. This research investigates the toxicity of three capping agents (carbonate, PVP, and citrate) to the luminescent marine bacteria *Vibrio fischeri*. The Microtox® basic acute toxicity test was used to determine the IC50s. Total silver and percent ionic silver of AgNP carbonate, AgNP citrate, AgNP PVP, and AgNO<sub>3</sub> were measured with a polymer membrane Ion Selective Electrode. The capping agents themselves were not toxic at the concentrations expected in the AgENP IC50s. The IC50s of the three AgENPs were compared to AgNO<sub>3</sub> as total silver and ionic silver. The toxicity ranking based on the IC50s, as total silver, was AgNO<sub>3</sub> > AgENP PVP > AgENP Citrate > AgENP CO<sub>3</sub>. When the toxicity was expressed as ionic silver, there was no difference between the AgNO<sub>3</sub>, AgNP PVP and AgNP Citrate, indicating that the ionic form of the AgENP is likely responsible for the observed toxicity to *V. fischeri*. The AgNP CO<sub>3</sub>, however, was less toxic than the AgNO<sub>3</sub>. Future work includes physical characterization of the AgENPs and investigations of whether Suwannee River humic acids modify the toxicity of these coated AgENPs.

**MP274 Monitor of Ion Dissolution from Ag Nanoparticles using Ion Selective Electrodes for the Understanding of Ag Nanoparticle Toxicity to *S. oneidensis*** M. Maurer-Jones, L. Chen, M. Mousavi, I.L. Gunsolus, C.J. Christenson, P. Buhlmann, C.L. Haynes, University of Minnesota. Increased use of silver nanoparticles in a variety of consumer products has elevated the potential of release and toxicity in the environment. Since Ag<sup>+</sup> ion is considered to cause toxicity, it is critical to determine ion dissolution from nanoparticles in ecologically relevant conditions. In this work, we

utilized ion selective electrodes with an ionophore-doped fluoruous phase membrane to selectively, sensitively, and dynamically measure the concentration of Ag<sup>+</sup> release from citrate-capped Ag nanoparticles over time in water and bacterial growth broth. Nanoparticles were characterized with TEM, zeta-potential, and UV-vis and stability of the nanoparticles were also monitored over time using dynamic light scattering. Additionally, bacterial toxicity studies, including minimum inhibitory concentration, viability and growth curve analysis, were performed with the bacteria *Shewanella oneidensis* to understand the toxicity of the nanoparticles and the relevant Ag<sup>+</sup> concentrations. Results indicate increased ion dissolution over time, though dissolution kinetics and concentration of released ions are similar between water and bacterial broth. Furthermore, Ag<sup>+</sup> ions cause changes in *S. oneidensis* growth and viability, which correlates strongly with Ag nanoparticle toxicity results, indicating Ag ion as a primary cause of nanotoxicity to *S. oneidensis*.

**MP275 The Influence of Dissolved Organic Carbon and Coating on the Toxicity of Silver Nanoparticles in *Daphnia magna*** K.M. Newton, Clemson University Institute of Environmental Toxicology / Biological Sciences, Clemson University / Clemson University Institute of Environmental Toxicology, Clemson University / Institute of Environmental Toxicology; S. Klaine, Clemson University / Department of Biological Sciences. Silver nanoparticles (AgNPs) are by far one of the most extensively used nanoparticles in general consumer products. However, the major concern regarding its prevalent use is the potential toxicity to aquatic organisms. The unrelenting question here is whether the toxicity is due to the AgNPs or the ions they release. The AgNP coating and the dissolved organic carbon (DOC) of the medium are important elements controlling silver ion release. They can influence the bioavailability and hence the toxicity of silver ions to aquatic organisms. This research investigated the role of these variables on AgNP toxicity to *Daphnia magna* and tested the hypothesis that toxicity could be explained by the ionic silver concentration. Forty-eight hour lethal median concentration (LC50) values were obtained using AgNO<sub>3</sub> (positive control) and three AgNPs with different surface coatings; Gum Arabic-coated (AgGA), polyethylene glycol-coated (AgPEG) and polyvinylpyrrolidone-coated (AgPVP) nanoparticles in moderately hard reconstituted water (MHW) alone or augmented with one of two different concentrations of Suwannee River dissolved organic carbon (SRDOC). Measurements of the total silver and the dissolved silver at the LC50 concentrations were determined by inductively coupled plasma-mass spectrometry. The dissolution behavior in MHW and SRDOC of the AgNPs at the LC50 concentration was mapped at different time intervals (0-48h). The findings indicated that in MHW, AgNO<sub>3</sub> (1.06 mg/L) was the most toxic to *Daphnia magna*, AgPVP (14.81mg/L) was the least toxic and AgGA (3.16 mg/L) and AgPEG (3.41mg/L) had similar toxicity. The dissolved silver concentration of the AgNPs in MHW at their respective total Ag LC50 concentrations was comparable to the ionic concentration of AgNO<sub>3</sub> at its LC50 concentration. These results suggested that the toxicity was explained by ionic silver concentration.

**TP001 Applicability of sorbents to predict the bioavailability of polar pesticides** M.C. Archer, Southern Illinois University / Department of Chemistry and Biochemistry; A.D. Harwood, Southern Illinois University; M. Lydy, Fisheries and Illinois Aquaculture Center and Department of Zoology. The importance of bioavailability in assessing the risk of environmental contaminants is well understood, and several techniques have been developed to evaluate bioavailability in sediments. Currently, a polymer resin, Tenax TA, has been used to measure desorption rates, which can subsequently be used to estimate bioavailability and toxicity of certain hydrophobic organic contaminants (HOCs). Although Tenax TA is useful for estimating the bioavailability of several legacy HOCs (log Kow >5), the applicability for use with more polar compounds was unknown. Furthermore, the potential of other sorbents to estimate bioavailability in sediments was largely unstudied. This study focused on the comparison of a new sorbent Diaion, to the commonly used Tenax TA, in their potential to estimate the bioavailability of pesticides including some with greater polarity. Diaion was selected because, like Tenax, it was easily separated from a water: sediment matrix and was suspected to have similar sorption capacity for HOCs. Based on the recommendations of the manufacturer, it was hypothesized that Diaion may be used for more polar compounds as well. Five pesticides that varied in polarity and water solubility were tested including tefluthrin, propiconazole, phostebupirim, atrazine, and chlorpyrifos. The ability of the two sorbents to remove these compounds from water, a surrogate for the unbound fraction, was tested. Additionally, the comparative ability of Diaion to measure the desorption of these compounds from sediment was evaluated. Both Diaion and Tenax have the potential to remove compounds of slightly greater polarity (low Kow < 5) with similar success (percent recoveries >80%), however, percent recoveries decreased substantially (60-80%) for atrazine (log Kow 2.6) regardless of sorbent. Having an alternative sorbent, Diaion, that was as effective as Tenax, could decrease sorbent cost by about 85%. However, additional research on the applicability of this sorbent for estimating bioavailability in soils or sediments is required.

**TP002 Application of silicone rubber passive sampling devices in screening for non- and semi-polar pesticides and herbicides in freshwater systems** E.S. Emelogu, The Robert Gordon University Aberdeen / Institute for Innovation, Design and Sustainability in research (IDEAS); P. Pollard, The Robert Gordon University Aberdeen / Institute for Innovation, Design and Sustainability in research (IDEAS); C. Robinson, L. Webster, Marine Scotland Science / Marine Laboratory; F. Napier, Scottish Environment Protection Agency (SEPA); C. McKenzie, The Robert Gordon University Aberdeen / Institute for Innovation, Design and Sustainability in research (IDEAS); C. Moffat, Marine Scotland Science / Marine Laboratory. Amongst the major chemical stressors in freshwater systems, pesticides and herbicides may contribute significantly to the ecological risk, exhibiting both acute and chronic toxic effects even at low aqueous concentrations. The traditional use of spot sampling in water quality monitoring is generally inadequate to identify and assess the potential impact of pesticides in aquatic ecosystems. Large volumes of water are required for analysis to achieve desired detection limits. In addition, spot sampling generally disregards the importance of the freely-dissolved aqueous concentrations as the source of the toxic stress and can miss episodic contamination events. Passive sampling devices (PSDs) have the potential to circumvent these challenges. PSDs sequester and pre-concentrate contaminants over an extended sampling period, enabling the determination of time weighted average (TWA) freely-dissolved concentrations of non-dietary bioavailable contaminants at very low concentrations. Most PSDs sample compounds of specific polarity ranges, however, silicone rubber (SR) PSDs have been shown to be robust, cost effective and to have affinity for organic compounds of diverse polarity, including semi-polar to non-polar pesticides. They are therefore ideal for preliminary identification and assessment of the potential impact of pesticides in aquatic systems. In this study, the potential use of SR-PSDs for identification and assessment of pesticides and acid/urea herbicides in freshwater was compared with the use of autosamplers. SR-PSDs were deployed for 54 days in three streams flowing through an agricultural catchment in North East Scotland, United Kingdom (UK). Extracts of the SR samplers and composite water samples were analysed for 83 pesticides and 26 acid/urea herbicides that were relevant to the catchment area. Of these, 39 pesticides and 13 acid/urea herbicides were detected and quantified in at least one of the SR samplers, indicating their occurrence in the catchment and likely availability to aquatic organisms. On the other hand, a total of 41 compounds were identified in the composite water samples, of which

only 10 compounds, mostly the acid/urea herbicides, were quantifiable. Additional detections by the SR-PSDs reflect lower limit of detection through larger volume of water sampled compared to the composite water samples. This study shows the potential of SR-PSDs for assessing the environmental relevance of these compounds.

**TP003 Effect of pH on accumulation of weak bases by fish: An evaluation of competing models for branchial uptake using diphenhydramine as a case study** n. d'lubac, Baylor University / Academia; B.W. Brooks, Baylor University / Environmental Science and Biomedical Studies, Baylor University / Department of Environmental Science; J. Berninger, Baylor University / Department of Environmental Science; K.A. Connors, Baylor University / Institute of Biomedical Studies, Baylor University / Department of Environmental Science; C.K. Chambliss, Baylor University; R.J. Erickson, U.S. EPA / Mid-Continent Ecology Division; J.W. Nichols, U.S. Environmental Protection Agency / ORD, NHEERL, Mid-Continent Ecology Division. Concern for potential effects of pharmaceutical products on fish and other aquatic biota continues to increase. Many of these compounds are weak bases that will ionize at pH values commonly encountered in environmental exposures. Because pH values vary substantially among systems, this ionizing behavior may be relevant for understanding chemical accumulation and effects. In this study we used diphenhydramine (DFH), a weak base, to evaluate pH effects on accumulation. The reported pKa value for DFH is ~9.0 while the log K<sub>OW</sub> value for the neutral form is ~3.3. Fathead minnows were continuously exposed to DFH in water at nominal pH values of 6.7, 7.7, and 8.7. Measured DFH concentrations in plasma and whole-body tissue samples were strongly pH-dependent, tending to increase with increasing pH. These data sets were subsequently modeled under two assumptions regarding chemical uptake at fish gills. The first model employs the Henderson-Hasselbach relationship, parameterized using bulk water pH, to predict branchial uptake of the neutral form. The second model, which is based on that given by Erickson and co-workers (2007, Environ. Toxicol. Chem., 25:1522-1532), accounts for acidification of the gill surface by excretion of metabolically-derived CO<sub>2</sub> and NH<sub>3</sub>, as well as the ability of ionized molecules to maintain high diffusion gradients for the neutral form across cell membranes. The first model failed to adequately describe observed accumulation under all simulated conditions. The second model accurately described observed accumulation, but only when the apparent log K<sub>OW</sub> value for the neutral form was increased to >4.0. In mammals, DFH is highly bound to plasma proteins, and it is possible that similar binding in fish is responsible for the modeled results. Ongoing studies are designed to characterize DFH binding in fish plasma.

**TP004 Legacy pollutant levels in crayfish compared to passive sampling devices: Determination of correlations and development of bioaccumulation models** N.D. Forsberg, Oregon State University / Environmental and Molecular Toxicology; G. Sower, ENVIRON International Corporation; K.A. Anderson, Oregon State University / Environmental & Molecular Toxicology, Oregon State University / Dept. of Environmental & Molecular Toxicology. Polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) are persistent organic compounds that continue to drive risk management efforts. In order to characterize exposure pathways and assess human health risks associated with consumption of contaminated resident fish, regulatory agencies conventionally analyze homogenized fish tissue samples for PAHs and PCBs. However, obtaining fish for analysis is challenging, the analysis is destructive, and the analysis offers limited spatial and temporal information due in part to biological and physiological variability. For these reasons, an alternative tool capable of accurately predicting fish tissue contaminant levels would be useful to regulatory agencies and risk managers. Passive sampling devices (PSDs) sample the bioavailable fraction of chemicals, are readily available in large numbers, provide a non-destructive sampling method, yield simpler chemical analyses, and are capable of identifying spatial and temporal contamination patterns. We hypothesize that PSDs are capable of providing site-independent predictions of chemical load in aquatic tissues with useful accuracy. To test this, PSDs were deployed in the water column at 8 different sites within and outside of the Portland Harbor Superfund Megasite for 24 days. Simultaneously, nearly 100 resident crayfish were collected. Sites selected for PSD and crayfish sampling represented a wide range of PAH and PCB concentrations. PSDs and crayfish were then analyzed for 16 priority pollutant PAHs and 32 dioxin and non-dioxin like PCB congeners using a novel analytical method. Presented results will include regression analyses on total contaminant loads (ΣPAH + PCBs),



chemical class specific loads ( $\Sigma$ PAHs,  $\Sigma$ PCBs), and individual contaminant of concern loads. Additionally, we will determine PSD-crayfish specific bioaccumulation factors ( $BAF_{\text{PSD-CF}}$ ) for a wide suite of contaminants of concern. PAH and PCB PSD loads will subsequently be used as surrogates for crayfish and used to generate human health risk estimates. Estimates will then be compared to risk results reported in the Portland Harbor Public health assessment for consumption of impacted resident crayfish. Results from this study will help identify novel PSD applications that could provide useful information to regulators and risk managers.

**TP005 Linkage between laboratory and field exposures of low-density polyethylene film as passive sampler in seawater** W. Lao, Southern California Coastal Water Research Project (SCCWRP); D. Tsukada, K. Maruya, SCCWRP. Due to its low material cost and large sorptive volume, low-density polyethylene (PE) film has gained popularity as a passive sampler for aqueous phase hydrophobic organic chemicals (HOCs). However, the accuracy and complexity involved with correcting for non-equilibrium exposure conditions remains an impediment for widespread acceptance. The goal of this study was to determine if correction using performance reference compounds (PRCs) could be simplified by relating the response of diverse classes of HOCs to a small number of PRCs. Kinetic uptake ( $k_1$ ) and release ( $k_2$ ) rate constants and equilibrium partition coefficients ( $K_{\text{pew}}$ ) were estimated for 96 individual HOCs (e.g. pyrethroids, PBDEs, organochlorine pesticides, PAHs and PCBs) in time-series seawater exposures and models for stable (e.g., PAHs) and unstable HOCs (e.g., most pyrethroids). Estimated log  $K_{\text{pew}}$  values showed good correlation with the logarithm of the octanol-water partition coefficient ( $\log K_{\text{OW}}$ ). Based on the relative values of modeled parameters and  $K_{\text{OW}}$  range, the kinetics of sorption was limited by diffusion within the PE film or by the aqueous diffusion boundary layer at the interface of the PE film. Identical  $k_2$  ratios between  $\text{C}^{13}$  labeled PRCs and their native analogs in lab and pre-loaded field exposed PE samplers suggested that a universal relationship for prediction of non-equilibrium correction factors is feasible. This relationship was applied to the estimation of seawater concentrations of pre-calibrated HOCs for PE samplers deployed in marine waters off the coast of southern California.

**TP006 Physiologically Based Pharmacokinetic (PBPK) Model for Residues of Diclofenac in Livestock and Vulture** X. Cao, University of Toronto / Chemical Engineering and Applied Chemistry; J. Armitage, University of Toronto Scarborough; F. Wania, University of Toronto Scarborough / Dept. of Physical & Enviro. Science, University of Toronto at Scarborough / Dept. of Physical & Enviro. Science. Veterinary use of diclofenac, a non-steroidal anti-inflammatory drug, has been implicated in the dramatic declines in vulture populations in South Asia observed over the past decade. Vultures can be exposed to the drug when they feed on carcasses of livestock that were treated with diclofenac before death. Here we develop and apply a dynamic Physiologically Based Pharmacokinetic (PBPK) model to simulate uptake, distribution, metabolism, and excretion of this ionizable organic compound (weak acid,  $\text{pK}_a \sim 4.0$ ) in vultures. The model consists of 10 compartments including gut tissue (main site of uptake), liver (main site of biotransformation) and kidneys (tissue exhibiting acute toxicological response). It is fugacity-based but has been modified so as to be broadly applicable to ionizable organic chemicals. For example, generic approaches are used to estimate the tissue-specific partitioning and distribution coefficients which determine the sorption capacity of the various compartments included in the model. Diclofenac residues in different tissues in cattle are used to define scenarios for the daily oral exposure of vultures. The PBPK model is then used to simulate how the compound behaves in vultures and to determine what daily oral dose is required to result in tissue residues in kidneys associated with acute toxicity (i.e. lethality). The simulated daily oral dose is then compared to empirical data to evaluate model performance.

**TP007 Two Applications of an Innovative Air Sampling Technology to Address Community-based Environmental Exposures to Legacy and Emerging PAHs** L.G. Tidwell, N. Forsberg, G. Wilson, Oregon State University / Environmental & Molecular Toxicology; J. Donatuto, Swinomish Indian Tribal Community / Office of Planning; S. Harris, Confederated Tribes of the Umatilla Indian Reservation / Department of Science and Engineering; K.A. Anderson, Oregon State University / Environmental & Molecular Toxicology. Although communities often want and need chemical monitoring data to characterize chemicals in their environment or from their activities, air monitoring equipment is often cost prohibitive

or technically impractical. We are further developing air monitoring bio-analytical tools that employ our passive sampling device (PSD). PSDs require no external power, require minimal training for quality controlled sampling, and can be quickly and inexpensively deployed. PSDs are capable of providing qualitative and quantitative characterization of exposure to the bioavailable vapor phase fraction of legacy and emerging contaminants in the atmosphere. We demonstrate our PSD's utility in two different scenarios; at the interface of community tribal lands and high intensity industrial activities, and in Native American fish smoking activities. Legacy and emerging PAHs were characterized for both scenarios. Communities adjacent to highly industrialized lands often want to understand the contribution of contaminants of concern from industrial activities to their ambient activities. The Swinomish Indian Tribal Community (SITC) offered their lands to further test our developing air PSD technology and to address these kinds of questions. PSDs were deployed at SITC, which borders a petroleum facility, for a yearlong study, from these PSD samples legacy and emerging PAHs were identified and quantified. The results indicate spatial and temporal trends that were related to the activities of the tribal community and the petroleum facility. While PSDs have been demonstrated as a surrogate of fish for aquatic environment assessment, atmospheric PSDs have not been previously used as a surrogate of food for preparation technique assessment. Community-based requests often include understanding the effects of special food preparations that are culturally important. The Confederated Tribes of the Umatilla Indian Reservation (CTUIR) requested an assessment of their fish smoking methods. As part of a larger study, we deployed air PSDs during the CTUIR smoking events to further evaluate the utility of using our PSD as a surrogate of smoke-processed fish. In a side-by-side study the PSDs were paired with salmon during smoking. PAHs were quantified from the air PSD and compared with the smoked salmon. The results show excellent promise for an alternative method for assessing food smoking preparation techniques.

**TP009 Characterization of Oil Dispersants and Their Effects on Petroleum Solubility and Volatilization** M. Azzam, University of Houston-Clear Lake; C.C. Zhang, University of Houston-Clear Lake, University of Houston-Clear Lake / Environmental Science. Dispersants are a mixture of surfactants and hydrocarbon-based solvents that disperse oil into droplets within the water column. Little data are available on the chemical compositions of commercial dispersants, whereas limited information also exists on the effects of these dispersants on solubility and volatilization processes of petroleum components in a mixture system. This study addressed these two areas of data scarcity needed to assess their environmental and ecological impacts from dispersant uses. Chemical components in three commercial dispersants were identified using an array of mass spectrometries (GC-MS, LC-MS-ESI and LC-MS-TOF). The effects of one dispersant on the fate and transport processes of oil components were further examined using batch studies under well-controlled conditions. GC-MS analysis showed the presence of 2-butoxyethanol, propylene glycol, di-propylene monobutyl and di-propylene glycol monomethyl ethers as well as a range of linear and branched aliphatic hydrocarbons ( $\text{C}_{10} - \text{C}_{30}$ ) and oleic acid methyl ester. LC-MS-ESI and LC-MS-TOF analysis showed the presence of alkyl polyethoxylated, amides and amines, Tween 80, and sulfosuccinate surfactants in dispersant. The commercial dispersant more effectively enhanced the aqueous solute solubility at low concentration than would conventional surfactants (SDBS and Tween 80) do at or above their critical micelle concentrations (CMCs). It was shown that the HLCs for the BTEX components in their pure form decrease in the presence of conventional surfactants (SDBS and Tween 80), but is not the case for the BTEX components in the mixed system. HLCs of the BTEX compounds in their pure form and in a multi-components system were found to be decreased significantly with the commercial dispersant. Pure surfactants impact the representative oil components at the CMCs and at higher concentration, while a little difference was noticed below the CMCs. In the mixture system, the pure surfactant aqueous solutions were found to deviate the vapor pressures, and HLCs from the ideal solutions due to the intermolecular interactions formed in these solutions. The implications of our results on the assessment of dispersant use and their short- and long-term effects (e.g., the effect of suppressed volatilization to atmosphere on other fate and transport processes of petroleum hydrocarbons) after oil spills will be discussed.



**TP010 Acute toxicity tests of airborne particles in filter extracts from air quality monitoring program of Vitoria city, Brazil** P.B. Miqueletto, S. Cassini, N. Reis, J. Santos, R. Keller, University of Espírito Santo. Vitória, ES, Southeast Brazil, is a coastal industrial city, which has been faced with increasing urban air pollution derived mainly from automotive fleet and robust steel making industries that are being appointed as main cause of respiratory diseases and allergy in certain municipality areas. The State Environmental Agency from Espírito Santo has an air pollution monitoring program of several areas within the municipality area with the purpose to identify the air pollution levels and sources. The main objective of this work was to develop a new approach to evaluate air pollution toxicity levels, by using air filters from the monitoring program through the Microtox acute toxicity analysis. The main hypothesis behind this approach is that the air filters can be the background aerial contamination which can be screened by the Microtox. The silica air filters derived from several points of the city were processed and the extraction was performed through a sonication of the filters generating aqueous extracts of main soluble fraction of dust and particles that have been retained. Results were expressed in EC<sub>50</sub> (Effective Concentration), the sample concentration value in which 50% of the bacterial luminescence is inhibited. Major toxic effects could be observed in two samples, collected in points from "Downtown" (FD) and "Suá" (FS). The first one presented an EC<sub>50</sub> of 148% after five minutes of contact with the bacterial culture and 111.3% in 15 minutes; and the latter 29.83% and 21.73% after five and 15 minutes, respectively. These data indicates that FS sample presented significant toxic effects, probably due to the particulate material and other pollutants that have occurrence in the collection point. The FD sample, in spite of a minor toxic effect observed in comparison with FS, also presented toxicity levels higher than other points from the city (data not shown) that cannot be neglected. Both points are located in areas near industrial activities. Preliminary analysis have detected levels of Na, Mg, Al, Si, S, Cl, C, Ca, Ti, V, Cr, Mn, Fe, Ni and Cu in the filters ranging from concentrations of 0,005 µg/m<sup>3</sup> (V) up to 39,33 µg/m<sup>3</sup> (Si). Further analyses related to chemical elements are in progress, in order to analyze the major sources of the toxicity observed. This new approach can be seen as additional tool to analyze and locate urban and industrial areas with active air pollution problems.

**TP011 Determination of Urban, Transition, and Rural Concentrations of Cyclic Siloxanes in Air Using a Low-Volume Active Sampling Method** R. Yucuis, University of Iowa / Civil and Environmental Engineering; C. Minner, University of Iowa / Statistics and Actuarial Science; K. Hornbuckle, University of Iowa / Civil and Environmental Engineering. The organosilicon compounds octamethylcyclotetrasiloxane (D4), decamethylcyclopentasiloxane (D5), and dodecamethylcyclohexasiloxane (D6) are high production volume chemicals that are widely used in household goods and personal care products. Due to their prevalence and chemical characteristics, cyclic siloxanes have been targeted for study as possible persistent organic pollutants. A simple and sensitive method for the sampling and analysis of the cyclic siloxane compounds D4, D5, and D6 in air has been developed and validated. Ambient air samples were collected in locations with varying population density in order to assess the potential impact of these compounds on humans and the environment depending on location type. Several indoor samples were also collected to compare levels and compound ratios to outdoor results. The indoor concentrations were very high compared to outdoor, with a median of 1770 ng m<sup>-3</sup> for the sum of D4, D5, and D6. Emissions were calculated using the indoor measurements and averaged 89 mg/day which is similar to that reported for North America in a recent modeling study. Outdoor sampling locations were over Lake Michigan near Chicago, in downtown Chicago, in Cedar Rapids, IA, and West Branch, IA, and had median sum siloxane levels of 300, 280, 73, and 29 ng m<sup>-3</sup> respectively. A diurnal trend is apparent in the samples taken at the IIT site in Chicago. Nighttime samples had a median 260 ng m<sup>-3</sup> higher than daytime samples. D5 was the dominant siloxane in both indoor and outdoor samples.

**TP012 Air measurements of linear and cyclic volatile methyl siloxanes along a transect between Portugal and Norway** A. Cincinelli, University of Florence / Chemistry Dept, Lancaster Environment Centre; F. Pieri, T. Martellini, University of Florence; N. Ratola, University of Murcia/University of Porto / Physics of the Earth; A. Katsoyiannis, Norwegian Institute for Air Research (NILU); S. Del Vento, J.K. Schuster, K.C. Jones, Lancaster Environment Centre. Linear and cyclic volatile methyl siloxanes (IVMSs

and cVMSs, respectively) are widely used in today's society, in particular in personal care products, cosmetics and industrial applications. These compounds are of environmental concern and currently under consideration for regulation because of their volatility, persistence, toxicity and tendency to bioaccumulate. Air measurements of l- and c-VMSs (including L2, L3, L4, L5, D3, D4, D5, and D6) were performed using sorbent-impregnated polyurethane foam (SIP) disk passive air samplers. Air samples were collected between February and September 2011 at 18 sites worldwide, along a rural/remote latitudinal transect from Portugal to Norway. Aim of this work was to evaluate the ground levels of organosilicon compounds at different sites worldwide; and to better address questions regarding distribution, sources, spatial pattern and chemical signature of siloxanes. The cyclic siloxanes occurred in all samples in significantly higher concentrations than the linear siloxanes. Concentrations of cVMSs ranged between 16.30 and 244.2 ng m<sup>-3</sup>, while lVMSs ranged between 1.13 and 58.61 ng m<sup>-3</sup>. Spatial distribution and correlation between VMSs were investigated in order to get insight on sources. Different siloxane profiles were observed between urban and background areas, evidencing the influence of different sources. The geographical pattern of all compounds reflected suspected regional emission patterns and highlighted localized hotspots, and spatial and temporal trends in background worldwide air, mainly reflecting the strength of primary diffusive emissions of these compounds.

**TP013 Atmospheric Polychlorinated Biphenyl Congeners and Synthetic Musk Fragrances in Chicago and Lake Michigan** Z.L. Rodenburg, University of Iowa, The University of Iowa / Dept. of Civil and Environmental Engineering; D. Hu, The University of Iowa / Department of Civil & Environmental Engineering, IIHR-Hydrosience and Engineering; K.C. Hornbuckle, University of Iowa / Department of Civil & Environmental Engineering, IIHR-Hydrosience and Engineering; Y. Ma, M. Venier, R. Hites, Indiana University / School of Public & Environmental Affairs. Polychlorinated biphenyl (PCB) concentrations in Chicago air near Lake Michigan declined rapidly following the production ban of Aroclors over 30 years ago. However, since the early 1990s, concentrations of these chemicals have decreased only slightly. Synthetic musk fragrances (SMFs), alternatively, are still used in many consumer products, although their atmospheric concentrations in urban and suburban areas are not particularly well characterized, spatially or temporally. In order to better understand contemporary atmospheric concentration trends for these two classes of chemicals in a large metropolitan area, an extensive passive air sampling network has been established and operating throughout Chicago and along an urban-rural gradient extending beyond its suburbs since December, 2010. Furthermore, during September, 2010, an active field sampling expedition was conducted on Lake Michigan aboard the EPA R/V *Lake Guardian* approximately 5 km off the coast of Chicago. Passive samples were collected on polyurethane foam discs (PUF), and high-volume active air and water samples were collected using Amberlite XAD-2 resin as an adsorption media and quartz fiber filters (QFF) to collect particles in both phases. Air samples were extracted via Accelerated Solvent Extraction (ASE) and water samples via Soxhlet prior to analyzing for 209 PCB congeners (sum of 158 peaks), six polycyclic musks (HHCB, AHTN, DPMI, ATIL, ADBI and AHMI) and two nitro musks (musk ketone and xylene) using gas chromatography tandem mass spectrometry (GC/MS/MS). Sample concentration data will be used to determine spatial and temporal variability among sampling sites, identify potential emission sources, and calculate instantaneous PCB congener fluxes across the air-water interface at the Lake Michigan location. The analysis also includes a comparison of concentration data for PCBs and SMFs with those for polycyclic aromatic hydrocarbons (PAHs) and organohalogen and organophosphorus flame retardants collected with duplicate samplers and analyzed at Indiana University.

**TP014 Influence of PCB-contaminated water bodies on the PCB content of riparian vegetation through foliar deposition** T.M. Sattler, Clemson University; V.D. Dang, University of Florida / Physiological Sciences; C.M. Lee, Clemson University / Environmental Engineering and Earth Sciences Department, Clemson University / Environmental Engineering and Science Department. Polychlorinated biphenyls (PCBs) are persistent organic pollutants (POPs) that tend to cycle between environmental compartments due to sorption to surfaces at lower temperatures and subsequent volatilization at higher temperatures. Evergreen plants, such as rhododendrons, have the potential to serve as biomonitors of atmospheric PCBs since they often retain foliage for several years. Volatilization of PCBs

from multiple sources (air, soil, water) of differing chiral signatures may result in an intermediate chiral signature in plants, alluding to the relative contribution various PCB sources may have on the overall PCB content in plants. We hypothesized that PCB-contaminated water bodies, such as Town Creek, Pickens, SC, will have a strong contribution to the PCB content of leaves on adjacent rhododendron plants through foliar deposition. Passive air samplers (PAS) will be deployed for a period of one month, located directly adjacent to each rhododendron plant at heights corresponding with the leaves to be sampled (lowest foliage available and ~1.5m). At the same time, polyethylene devices (PEs) will be deployed in Town Creek near rhododendron plants to measure dissolved PCBs. Rhododendron leaf and soil samples at increasing distance from the stream will be collected in conjunction with the PAS and PE. Samples will be analyzed using chiral gas chromatography. Preliminary results show higher total PCB concentrations in leaves collected during the fall and winter ( $4153.4 \pm 601.6$  and  $3722.2 \pm 93.3$  ng/g lipid, respectively) than in spring ( $727.4 \pm 199.5$  ng/g lipid). Total PCB concentrations in rhododendron leaves and PAS are expected to decrease with increasing distance away from the surface water. PCBs measured in Town Creek and the closest rhododendron leaves sampled are expected to have similar homolog distributions and chiral signatures. PCBs measured in rhododendron leaves sampled furthest from the creek may have significantly different homolog distributions and chiral signatures if another atmospheric PCB source is present, although this is not expected to be significant.

**TP015 Air concentrations of PCBs and OC pesticides using PUF-disks passive air samplers in Sicily Island, Mediterranean Region** K. Pozo, Masaryk University / RECETOX; T. Harner, Environment Canada / Atmospheric Science & Technology Directorate, Environment Canada / ASTD; J. Klanova, Masaryk University / RECETOX; S. Corsolini, University of Siena / Dept Environmental Science. Passive air samplers (PAS) have become an increasingly popular and cost-effective technique for assessing air concentrations of pesticides. Air concentrations ( $\text{pg m}^{-3}$ ) of PCB and OCP were measured in coastal areas of Sicily Island using PAS from July to October 2007. Results shows that PCB were detected in all sites and concentrations ( $\text{pg m}^{-3}$ ) ranged from 5 to 153 ( $47 \pm 54$ ). As expected, the highest concentrations were detected at urban sites and the lowest concentrations were found at background sites. PCB congener profiles show a predominance of certain classes: tri-Cl (PCB-18, -17, -16+32, -28+31, -33), tetra-Cl (PCB-42, -44, -49, -52, -66, -70), hexa-Cl (PCB-138, -153) and hepta-Cl (PCB-180). Highly chlorinated PCBs (hexa and hepta-Cl) were predominant at urban/industrial centres (Sferracavallo and Milazzo) while less chlorinated PCBs (tri- and tetra-Cl) were more abundant in low impact areas. From individual PCB congener, PCB-28 was the most predominant detected at all sampling sites. For OCPs, HCHs concentrations ( $\text{pg m}^{-3}$ ) were relatively high, in the range 50-1034  $\text{pg m}^{-3}$  ( $312 \pm 323$ ) for  $\alpha$ -HCH. Winds in these areas are prevalently northerlies (Maestrale, Grecale and Tramontana) with some southerlies (Scirocco, Meridione and Libeccio) which blow from the land and may contribute with pesticides. PAS are useful techniques for time integrated monitoring studies.

**TP016 Simulating and explaining passive air sampling rates for semi-volatile compounds on polyurethane foam (PUF) disk** N.T. Petrich, University of Iowa / Environmental Engineering – CGRER; S.N. Spak, University of Iowa / Public Policy; G.R. Carmichael, University of Iowa / Chemical and Biochemical Engineering; D. Hu, A. Martinez, K.C. Hornbuckle, University of Iowa / Environmental Engineering. Polyurethane foam (PUF) passive air samplers are widely deployed as an inexpensive and practical way to sample semi-volatile pollutants. However, concentration estimates from passive sampling to date have relied on estimating constant empirical mass transfer rates, which add unquantified uncertainties to concentrations and any spatial and temporal information they contain. Here we present a method for modeling hourly sampling rates, mass transfer, and concentrations from hourly meteorology using first principle chemistry, physics, and fluid dynamics. This approach provides a way to quantify and explain the observed effects of meteorology on spatial and seasonal variability in congener-specific sampling rates and analyte concentrations; to assess PUF saturation; and to recover average concentration at a reference temperature. Modeled sampling rates are evaluated for gas-phase polychlorinated biphenyls (PCBs) at an urban network of seven flying saucer samplers in Chicago, Illinois during 2008 using local meteorological observations and those simulated by the Weather Research and Forecasting (WRF) model at

urban to regional scales. Modeled sampling rates are compared with results from depuration compounds, and temporal trends in analyte concentrations evaluated through comparison with active samplers. The model simulates average sampling rates within 17.2 ( $\pm 16.4$ ) % of those determined from depuration compounds. Results highlight that sampling rates are highly variable at hourly and daily scales, sensitive to spatial and temporal resolution in meteorology, and strongly congener-dependent, with predictable relationships between congeners. Both modeled and depuration-based sampling rates for low molecular weight congeners differ from high molecular high congeners by >16% consistently throughout the year, indicating the need to use congener-specific sampling rates when determining concentrations from passive samplers. We quantify the importance of each simulated process to sampling rates and mass transfer, and assess uncertainty contributed by each process, including advection, molecular diffusion, volatilization, and turbulent and laminar flow regimes within the sampler housing. Through this method, hourly congener-specific sampling rates, analyte concentrations, and PUF uptake can be simulated anywhere, at any time, from observed or modeled hourly meteorology.

**TP017 Evaluation of polyurethane passive samplers as a tool for occupational PAH measurements** B. Strandberg, University of Gothenburg / Occupational and Environmental Medicine; M. Lewné, Karolinska Institutet / Institute of Environmental Medicine; A. Wierzbicka, Lund University / Ergonomics and Aerosol Technology; G. Sallsten, University of Gothenburg / Occupational and Environmental Medicine. Exposure to PAHs at workplaces may give an increased risk of cancer. Routine monitoring of workers' exposure to PAHs in occupational environments are therefore fundamental. Best risk assessment is done using personal sampling. Monitoring of PAHs is mainly studied with active sampling in workplaces. However, to simplify routine monitoring, passive air samplers (PAS) may be the only option since they are cheap, easy to handle, and less disturbing. Disadvantages of active samplers are a big reason to the lack of studies of PAHs in occupational air. The use of polyurethane foam (PUF) as a PAS in outdoor ambient air is well accepted and its capacity has been quite well studied. Since the environmental conditions in occupational environments differ compared to outdoor the PUF may behave differently when deployed here. The PUF sampler needs to be further developed for typical occupational scenarios (i.e. 8 h exposure time and small enough for personal sampling). In addition, the PUF's ability to sequester particle associated PAHs has to be validated. The last is crucial since the most important PAHs concerning health effects (i.e. benzo(a)pyrene-B(a)P) is almost entirely associated to particles. Recently, we tested the PUF (cylinder design, total surface area =  $70 \text{ cm}^2$ ) in two occupational settings known to have elevated levels of PAHs. The results demonstrated that the PUF has a big potential for being used as a monitoring technique for both gas- and particle PAH exposure under a working day. The applicability and capacity of the PUF needs, however, to be further validated and tested under both laboratory and field conditions. In this work we tested the PUF, and determined uptake rates, in two exposure chamber experiments. In the first experiment wood smoke and the second diesel exhaust was generated. The concentrations in all these experiments ranged for instance for phenanthrene from 10 to 450  $\text{ng m}^{-3}$  and for B(a)P from 0.5 to 20  $\text{ng m}^{-3}$ . The uptake rates after 3-8 h sampling time for the 3-4 ringed PAHs were between 1.5 and 4  $\text{m}^3/\text{day}$  and for 5-6 ringed PAHs 0.2-0.8  $\text{m}^3/\text{day}$ . Following laboratory studies, the PUF were also tested in the field. In this study the PUF was deployed as personal and stationary samplers together with active low-volume samplers for a working day (8 h) in restaurant kitchens. The results show that the PUF has a good potential to be used as stationary or personal sampler to monitor the exposure to PAHs also at workplaces.

**TP018 Spatial and Temporal Trends of Neutral Polyfluoroalkyl Substances in the Global Atmosphere** A.B. Gawor, University of Toronto / Departments of Chemistry and Physical and Environmental Sciences; C. Shunthirasingham, University of Toronto Scarborough / Departments of Chemistry and Physical and Environmental Sciences; Y. Lei, University of Toronto Scarborough / Departments of Chemistry and of Physical and Environmental Sciences; T. Gouin, Unilever / Safety and Environmental Assurance Centre; S. Lee, Environment Canada / Atmospheric Science and Technology Directorate; T. Harner, Environment Canada / Atmospheric Science & Technology Directorate, Environment Canada / ASTD; F. Wania, University of Toronto Scarborough / Dept. of Physical & Enviro. Science, University of Toronto at Scarborough / Dept. of Physical & Enviro. Science.

Over the last decade, per and polyfluoroalkyl acids have been receiving widespread attention for their ubiquitous nature in the global environment and biota. Some volatile polyfluoroalkyl substances (PFAS), namely fluorotelomer alcohols (FTOHs), fluorinated sulfonamides (FOSAs), and fluorinated sulfonamidoethanols (FOSEs), have been implicated in facilitating the transfer by atmospheric long range transport to remote regions, where they may degrade either abiotically or biotically into the polyfluoroalkyl acids. As part of the Global Atmospheric Passive Sampling (GAPS) study, the temporal and spatial distributions of these volatile PFAS in the global atmosphere were investigated, giving for the first time insight into the interannual time trends of PFAS in the atmosphere on a truly global scale. Between 2006 and 2010, XAD-resin based passive air samplers were deployed annually at between 22 and 46 sites on six continents, ranging in character from polar to remote to urban. Higher levels of FTOHs compared to FOSAs and FOSEs were observed at all sites. Urban sites had the highest levels of PFAS compared to rural and polar sites, which is also apparent in a positive correlation of the levels with the proximity of the sampling sites to areas of suspected emissions (as quantified with the pertingency index). Levels of FOSAs and FOSEs tended to decrease steadily during the five years of measurements, whereas an initial decline in the concentrations of FTOHs from 2006 to 2008 did not continue in 2009 and 2010.

**TP019 Measurement of Airborne PCBs in East Chicago, IN and Columbus Junction, IA** D. Hu, University of Iowa / Chemical and Biochemical Engineering, The University of Iowa / Department of Civil & Environmental Engineering, IIHR-Hydroscience and Engineering; T. Schulz, The University of Iowa / Department of Civil & Environmental Engineering, IIHR-Hydroscience and Engineering; P.S. Thorne, The University of Iowa / Occupational and Environmental Health; J. DeWall, The University of Iowa / Department of Occupational and Environmental Health; K.C. Hornbuckle, University of Iowa / Department of Civil & Environmental Engineering, IIHR-Hydroscience and Engineering. Indiana Harbor and Ship Canal (IHSC) of East Chicago lies in a heavily industrial area of southern Lake Michigan and is known to be highly contaminated with PCBs and other pollutants. A dredging and disposal project of IHSC will place  $3.5 \times 10^6$  m<sup>3</sup> of sediment into a confined disposal facility (CDF). The close proximity of the CDF to houses and public schools causes concern of airborne PCB exposure to the surrounding population especially school students. Air samples are collected using polyurethane foam based passive samplers (PAS-PUF) and high volume air samplers (Hi-Vol) deployed in two communities at East Chicago, Indiana and Columbus Junction, Iowa. Columbus Junction is a rural community that has no known sources of PCBs. The air samples collected in the two communities were extracted and analyzed for the full suite of PCBs using GC/MS/MS. The PCB magnitude and profiles prior to the dredging activities are measured at East Chicago, and the results for schools and homes are compared between the two communities and to air samples collected immediately next to and over the IHSC water. The dredging is expected to start in summer of 2012, and our ongoing monitoring project is designed to assess impact of the dredging on human exposure to PCBs at the surrounding area.

**TP020 Size-Dependent Dry Deposition of Airborne Semivolatile Organic Compounds** K. Zhang, E.Y. Zeng, Guangzhou Institute of Geochemistry / State Key Lab of Organic Geochem.; S. Li, Environment Canada; C.S. Wong, University of Winnipeg / Environmental Studies Program and Department of Chemistry, Richardson College for the Environment; B. Zhang, Henan University of Technology; L. Zhang, R. Staebler, Environment Canada. The fate of atmospheric semivolatile organic compounds (SVOCs) is influenced by dry deposition. Despite the abundant efforts in characterizing dry depositions of SVOCs, quite a few knowledge gaps still exist, including limitation of surrogate surfaces, associated uncertainty from empirical deposition velocity, insufficient consideration of meteorological factors, and bias with measurements caused by locally generated resuspended particles, etc. To fill these gaps, we measured the dry deposition dynamics of SVOCs on a broadcast tower in urban Guangzhou (China) using a size-segregated sampling method and dry deposition modeling, which accounted for the influences of meteorological factors but without the confounding surface effects. Size-segregated particulate polycyclic aromatic hydrocarbons (PAHs) and polybrominated diphenyl ethers (PBDEs) were measured at the 100- and 150-m decks of the broadcast tower in daytime and at night in August and December 2010. Particulate PAHs and PBDEs were more abundant at night than in daytime, and in

winter than in summer, likely due to varying meteorological conditions and atmospheric boundary layers. More than 60% of particulate PAHs and PBDEs were contained in particles with an aerodynamic diameter ( $D_p$ ) below 1.8  $\mu$ m, indicating the effects of long-range transport. Based on the modeled size-dependent dry deposition velocities, the average daily particle dry deposition fluxes of PAHs ranged from 604 to 1190 ng/m<sup>2</sup>/d, and those of PBDEs ranged from 2.6 to 122 ng/m<sup>2</sup>/d. Deposition fluxes of all PAHs and PBDE congeners were significantly higher in daytime than at night for both sampling periods, due to the effects of diurnal variability in meteorological factors. Dry deposition velocities of PAHs and PBDEs were lower at night than those in the daytime, probably reflecting higher mechanical and thermal turbulence during daytime. Finally, disequilibrium phase partitioning, meteorological conditions and atmospheric transport were regarded as the main reasons for the variances in dry deposition velocities of individual PAHs and PBDEs. The present results indicate that caution should be exercised in selecting an appropriate value for dry deposition velocity for determining dry deposition of SVOCs.

**TP021 Urban Outflow Study of Polybrominated Diphenyl Ethers and the Bulk Carbon Fraction from Fort Worth, TX** A. Clark, Baylor University / Department of Chemistry and Biochemistry; P. Nallathambi, R.J. Sheesley, Baylor University / Department of Environmental Studies; S. Usenko, Baylor University / Department of Environmental Science, Baylor University / Department of Chemistry and Biochemistry. Texas has experienced one of the fastest growing populations in the United States. The Dallas and Fort Worth metropolitan areas include ~4.2 million inhabitants and has seen a ~20% increase in population from 2000 to 2010. Fort Worth has experienced a 38.6% increase in population during this past decade. An investigation of Fort Worth's urban outflow was performed in June 2011, when daily air samples were taken in a month long sampling campaign at the TCEQ Eagle Mountain Lake Site, 31 km downwind of downtown Fort Worth, TX. Total suspended particulate (TSP) air samples were analyzed for polybrominated diphenyl ethers (PBDEs) (including PBDE-28, 47, 99, 100, 153, 154, and 183) as well as organic and elemental carbon (OC and EC). Using a Tisch high-volume air sampler, approximately 20 TSP samples were collected for analysis on 20x25 cm quartz fiber filters at peak traffic times and overnight, sampling 160-480 m<sup>3</sup> of air over 4-12 hour intervals. Samples were spiked with isotopically labeled surrogates and extracted using accelerated solvent extraction techniques. Extracts were blown down to ~450  $\mu$ L and spiked with isotopically labeled internal standards before analysis via gas chromatography-electron capture negative chemical ionization mass spectrometry. EC and OC were measured on 1.5 cm<sup>2</sup> filter punches using a Sunset Laboratory Carbon Analyzer. EC and OC concentrations were 0.70 to 0.94 and 0.90 to 10, respectively. Relationships between PBDE, EC, and OC concentrations were examined along with sampling times and boundary layer height.

**TP022 Congener Specific Flux Estimation from Polychlorinated Biphenyl Contamination in Soil and Sand Surface Layers** B.J. Grigg, W. Mills, Mills Consulting, Inc.; L.J. Thibodeaux, Louisiana State University / Gordon A. & Mary Cain Dept. of Chemical Engineering. Accurate, theory-based mathematical algorithms for flux estimation of PCBs (and other SVOCs) from soils/sands are still somewhat lacking. As such, algorithms were introduced and verified on a bench-scale soil-to-air microcosm chamber measurements using congener-specific volatilization of Polychlorinated Biphenyl (PCB). The soil-to-air partitioning aspects of these algorithms differ for PCB loadings on contaminated soil and sand due to the much lower organic fraction of sands. Although developed for PCBs, this theory-based approach may be applied to any semi-volatile organic compound (SVOC) vaporizing from surface layers. In this work, the individual PCB congeners are treated as separate SVOCs and thus estimates can be made of the volatilization flux to air from theoretical models based on the individual congener loading in surface soils. Using these methods, the actual flux above a soil can be estimated by measuring the soil surface area (m<sup>2</sup>/g), temperature, wind speed, soil moisture content, soil organic content, particle size distribution, and SVOC loading on the soil. For both the soil and sand flux estimation algorithms, the predicted values correlated well with the observed fluxes from the bench-scale samples. Using a power fit, the R<sup>2</sup> statistic for the correlation between the predicted and bench-scale fluxes was 0.8976 (N=287) for the soil samples. This fits better than a linear trend line, which gives an R<sup>2</sup> of 0.8697. For the sand samples, the power fit R<sup>2</sup> statistic for this correlation is 0.8749 (N=262). A linear fit yields an R<sup>2</sup> of 0.6748, again



lower than a power fit. It is possible that the power fit has a higher  $R^2$  due to the non-linear nature of the equations used for the mass transfer coefficient and/or the soil organic carbon-water partitioning coefficient. Overall this work indicates that these methods are very useful in estimating flux from SVOCs in surface layers.

**TP023 Role of Wet Deposition in Transporting PAHs and n-Alkanes from Atmosphere to Ground: A Case Study in Guangzhou of South China** L. Guo, Guangzhou Institute of Geochemistry / State Key Laboratory of Organic Geochemistry; E.Y. Zeng, Guangzhou Institute of Geochemistry / State Key Lab of Organic Geochem.. Rainfalls for the entire 2010 were sampled simultaneously at three locations in Guangzhou, a mega-metropolitan center in South China, with the objective of assessing the importance of wet deposition in transporting polycyclic aromatic hydrocarbons (PAHs) and n-alkanes from atmosphere to urban environment. Concentrations of  $\Sigma_{22}$ PAH ranged from 20-890 ng/L and 2.4-160  $\mu\text{g/g}$ , respectively, in the filtrate and particulate samples. In addition, concentrations of filtrate and particular n-alkanes were in the ranges of 48-59000 ng/L and 62-1900  $\mu\text{g/g}$ , respectively. In terms of phase distribution, more than 80% of PAHs was associated with the particulate phase, while more than 77% of n-alkanes were affiliated with the dissolved phase. There was apparent spatial variability in wet deposition fluxes of PAHs and n-alkanes among the three sampling locations, reflecting different anthropogenic impacts from the regions of various extents of urbanization and weak atmospheric advection in general. The annual wet deposition fluxes of PAHs and n-alkanes to Guangzhou were 0.40 and 15  $\text{mg/yr m}^2$  for PAHs and n-alkanes and, respectively. The wet deposition flux of PAHs obtained in the present study was comparable to those acquired in Massachusetts of the United States (0.29  $\text{mg/yr m}^2$ ) and Hungary (0.24 and 0.4  $\text{mg/yr m}^2$ ) and lower than that (1.6  $\text{mg/yr m}^2$ ) in Turkey. In comparison, dry deposition fluxes of PAHs and n-alkanes were 0.26 and 5  $\text{mg/yr m}^2$ , respectively, similar to the dry deposition fluxes. This indicated that wet deposition played an important role in removing PAHs and n-alkanes from the atmosphere of Guangzhou.

**TP024 Transport and Fate of Chiral and Achiral Organochlorine Pesticides in the North Atlantic Bloom Experiment** L. Zhang; T.F. Bidleman, Umea University / Chemistry Department; L.J. Thibodeaux, Louisiana State University / Gordon A. & Mary Cain Dept. of Chemical Engineering; M. Perry, Ira C. Darling Marine Center; R. Lohmann, University of Rhode Island / Graduate School of Oceanography, University of Rhode Island. Organochlorine pesticides (OCPs) were measured in the surface seawater and lower atmosphere during the North Atlantic Bloom Experiment in spring 2008 on the *R/V Knorr*. The gaseous concentration profiles resulted from both long range transport (LRT) from the Arctic by polar easterlies and local biogeochemical processes. Relatively constant  $\alpha/\gamma$ -hexachlorocyclohexane (HCH) ratios and enantiomer fractions (EFs) of  $\alpha$ -HCH indicated that a single water mass was sampled throughout the cruise. Changes in dissolved phase concentrations were dominated by bloom processes (air-water exchange, partitioning to organic particles, and subsequent sinking) rather than LRT.  $\alpha$ -HCH and dissolved phase trans-chlordanes showed depletion of (+) enantiomer, whereas depletion of the (-) enantiomer was observed for heptachlor *exo*-epoxide (HEPX) and cis-chlordanes. Fugacity ratio calculations suggest that hexachlorobenzene (HCB) and  $\gamma$ -HCH were depositing from air to water whereas heavier OCPs (chlordanes, HEPX) were evaporating. Dissolved phase concentrations did not decrease with time during the three-week bloom period; neither were lipophilic OCPs drawn down from air to water as previous studies hypothesized. Comparison with Arctic measurements suggested that the Arctic returned higher concentrations of  $\alpha$ -HCH and HCB through both the atmospheric (polar easterlies) as well as oceanic transport (East Greenland Current) to the lower latitudes. An Eulerian box model was developed to simulate the dissolved phase concentrations time series profiles and compare with the observed values, which allow us to examine our scientific understanding of processes that affect the aquatic fate of OCPs during the bloom and evaluate the relative significance of each process.

**TP025 The importance of chlorine as an atmospheric oxidant for organic pollutants** C.J. Young, Memorial University of Newfoundland / Department of Chemistry, Memorial University. Typically, the impact of chlorine on the atmospheric fate of organic compounds has been thought of as negligible on a global scale, where only a small effect of chlorine oxidation would be expected in the marine boundary layer. Recently, it has been

shown that chlorine atoms can dominate the atmospheric fate of volatile organic compounds during ozone formation bursts in the Arctic. Further, the presence of chlorine has been detected in mid-continental regions, far from oceanic chloride sources. The chlorine atom is a much more powerful oxidant than the dominant oxidant in the atmosphere, hydroxyl radical. Thus, for slow-reacting compounds, oxidation by chlorine atoms rather than hydroxyl radicals could significantly impact their fate. For example, the rate constant for reaction of fluorotelomer alcohols (the atmospheric precursor to persistent perfluorocarboxylic acids) with chlorine is fifteen times faster than with hydroxyl radical. Using the Master Chemical Mechanism model and examples of volatile organic compounds and slow-reacting compounds, the role of chlorine atom oxidation on the fate of organic pollutants will be discussed.

**TP026 Effects of Aryl Hydrocarbon Receptor Genetic Variants on Dioxin-induced Transactivation in MRL/lpr and C3H/lpr Mouse Strains** S. CHO, Kyung Hee University / Department of Life and Nanopharmaceutical Science, Department of Life and Nanopharmaceutical Science, and Department of Biology; Y. Miura, K. Suzuki, Ehime University / Center for Marine Environmental Studies (CMES); T. Miyazaki, M. Nose, Ehime University / Department of Pathogenomics; H. Iwata, Ehime University / Center for Marine Environmental Studies; E. Kim, Kyung Hee University / Department of Biology. The aryl hydrocarbon receptor (AHR) is activated by a variety of chemicals, including dioxin-like compounds (DLCs), and participates in the transcriptional regulation of some xenobiotic-metabolizing enzymes such as cytochrome P450 1A1 (Cyp1a1). It is known that an MRL/lpr strain of mice develops various types of autoimmune diseases under the control of polygenes, while a C3H/lpr strain shows less severe diseases due to its distinct genetic background. We confirmed that the 375th amino acid residue in AHR that is responsible for dioxin susceptibility is Ala in a C3H/lpr strain, which is the same as that in a dioxin sensitive C57BL/6J strain. In contrast, the corresponding amino acid residue is Val in an MRL/lpr strain, which is the same as that of a dioxin resistant DBA/2 strain. To compare AHR-mediated responses to DLCs between C3H/lpr and MRL/lpr strains, the AHR expression plasmid and the Cyp1a1 5'-flanking region-containing reporter plasmid of each strain were transiently transfected in COS-7 cells, and the cells were treated with graded concentrations of 4 selected congeners. Results of the in vitro reporter gene assay showed that both AHRs had dose-dependent responses for all the congeners. For C3H/lpr AHR, EC50 values of 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, 2,3,7,8-TCDF and 2,3,4,7,8-PeCDF were estimated to be 0.023, 0.040, 0.050 and 0.18 nM, respectively. The TCDD-EC50 for C3H/lpr AHR was as low as that for C57BL/6J AHR, suggesting that a C3H/lpr strain may be sensitive to the exposure of DLCs as a C57BL/6J strain. This is supported by the result indicating the same Ala-type AHR in both C3H/lpr and C57BL/6J strains. On the other hand, EC50 values of 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, 2,3,7,8-TCDF and 2,3,4,7,8-PeCDF for MRL/lpr strain were 0.11, 0.11, 1.1 and 0.23 nM, respectively. Although MRL/lpr AHR may be less sensitive to the tested congeners than C3H/lpr AHR, the difference in TCDD-EC50 values between AHRs from MRL/lpr and C3H/lpr was unexpectedly small, because a larger difference in TCDD-EC50 values (0.29 nM for MRL/lpr and 0.019 nM for C3H/lpr) was observed between TCDD-treated splenocytes from the two strains. This implies that factors other than AHR may be involved in dioxin susceptibility.

**TP027 The Aryl Hydrocarbon Receptor Signaling Pathway of Fishes: Implications for Sturgeon Sensitivity to Dioxin-like Compounds** J.A. Doering, University of Saskatchewan / Toxicology Centre, University of Saskatchewan; S.B. Wiseman, University of Saskatchewan / Toxicology Centre, University of Saskatchewan / Post-Doctoral Fellow; S. Beitel, University of Saskatchewan / Toxicology Centre; J.P. Giesy, University of Saskatchewan / Biomedical Veterinary Sciences & Toxicology Centre, Michigan State University / Department of Animal Science, University of Saskatchewan / Toxicology Centre; M. Hecker, University of Saskatchewan / Toxicology Centre. Activation of the aryl hydrocarbon receptor (AhR) regulates all known effects of exposure to dioxin-like compounds (DLCs). Exposure to DLCs causes a range of adverse effects in fishes, including teratogenicity, hepatotoxicity, and reproductive impairment. Sturgeons are ancient species of fish and due to their endangered status are of interest for ecological risk assessment. This is particularly true in context with the exposure to bioaccumulative contaminants such as DLCs due to the longevity and benthic life-style of sturgeons. However, little is currently known about the AhR

signaling pathway of these or other ancient fishes. To characterize the sensitivity of sturgeons to DLCs we investigated the biochemical and molecular responses of white sturgeon (WS; *Acipenser transmontanus*) to a model AhR agonist,  $\beta$ -naphthoflavone (BNF). WS were found to be among the more responsive of fishes with regard to inducibility of CYP1A. Nucleotide sequences from three distinct AhRs (AhR1, AhR2, AhR3) were identified for the first time in WS by use of Illumina sequencing and RACE-PCR. WS AhR1 has closest identity to mammalian AhRs, WS AhR2 has closest identity to piscine AhR2s, and WS AhR3 has closest identity to avian AhR1s. All three identified AhRs had greatest expression in liver, gill, and heart tissues of WS. Following exposure to BNF, AhR2 and AhR3 expression was up-regulated in liver (6-fold and 5-fold, respectively), gill (10-fold and 5-fold, respectively), and intestine (35-fold and 2-fold, respectively); however AhR1 was only up-regulated in gill (5-fold). The functional significance of sturgeons expressing three AhR genes and implications for sturgeon sensitivity to DLCs is currently unclear. However, the great sensitivity of salmonids to DLCs is hypothesized to be partially driven by these species expressing multiple, functional AhR genes. Further research is currently ongoing to investigate the toxicological significance of the unique AhR signaling pathway in WS and to determine how this knowledge could be used to predict the relative sensitivity of other species of endangered sturgeons which are not easily acquired for toxicity studies.

**TP029 Regulation of Peroxisomal Proliferator Activated Receptors (PPAR) by Nitroaromatic Compounds** D.R. Johnson, U.S. Army Engineer Research and Development Center / Environmental Laboratory, U.S. Army / CEERD-EP-R, US Army Engineer Research and Development Center / Environmental Laboratory, US Army Engineer Research & Development Center / Environmental Laboratory; C. Ang, Badger Technical Services / Environmental Laboratory; E.J. Perkins, US Army Engineering Research & Development / Environmental Laboratory, US Army ERDC / Environmental Processes & Effect Division, US Army Engineering Research & Development / Environmental Genomics and Genetics Team.

PPAR nuclear receptors are intimately involved in regulating energy pathways. Previous studies demonstrated that nitroaromatic munitions compounds, but not cyclic nitramine munitions compounds, consistently inhibited the activation of the PPAR isoforms alpha and delta. This study build upon these findings by examining the effects of other therapeutic and xenobiotic nitroaromatics on PPAR activation. PPAR alpha, PPAR gamma, and PPAR delta activation was measured according to the cell-based nuclear receptor activation kits (Indigo Biosciences, State College, PA). Briefly, flutamide, 2-nitrofur, 2-nitroimidazole, nitrobenzene, aniline, and musk ketone (0, 0.1-0.3, 1, 3, 10, 30, and 100 mg/l) were added to wells containing transfected cells containing high levels of PPAR alpha, gamma, or delta. After 24 h, exposure, levels of nuclear receptor activation were measured by luciferase assays. Cytotoxicity was measured in HepaRG hepatocyte cell lines using the Neutral Red uptake method. Most of the studies compounds, with the exception of 2-nitroimidazole and aniline, showed increased cellular toxicity at concentrations above 10 mg/l. PPAR alpha activation was suppressed by flutamide, nitrofur, musk ketone, and 2,4-dinitrophenol (24, 63, 62, and 40% of control levels at 10 mg/l). This was similar to what was previously shown with nitroaromatic munitions (e.g., 2,4,6-trinitrotoluene [TNT]). In contrast, PPAR gamma activation was increased by flutamide, nitrofur, and musk ketone (174, 132, and 33% of control levels at 10 mg/l). PPAR delta activation was inhibited by most of the nitroaromatics tested, with the most potent being flutamide and 2,4-dinitrophenol (7 and 30% of control levels at 10 mg/l). These PPAR activation profiles were very similar to what was previously reported with nitroaromatic munitions and breakdown products (e.g., 2,4,6-trinitrotoluene [TNT] and 2-amino-4,6-dinitrotoluene [2-ADNT]). These data suggest that nitroaromatic compounds may have a common mechanism of action of inhibiting PPAR alpha and delta nuclear receptors. This ligand-based regulation of nuclear receptor activity may result in alterations of energy molecule production and energy regulation in affected organisms.

**TP030 Risk-based water quality assessment through bioanalytical tools**

A. Poulsen, Entox, The University of Queensland / National Research Centre for Environmental Toxicology; F. Leusch, H. Chapman, Griffith University, Smart Water Research Centre; B.I. Escher, Entox, The University of Queensland / National Research Centre for Environmental Toxicology. A multitude of organic contaminants including agricultural and industrial chemicals, as well as, pharmaceuticals and personal care products enter the

aquatic environment to form complex mixtures. The ever-increasing number of chemicals circulating in the environment and the increasingly limited water resources have increased demands to assess the quality of waters used for human consumption and recreation. Target chemical analysis efficiently identifies selected pollutants but may overlook non-target compounds including potentially toxic metabolites, emerging and unknown chemicals. Furthermore, while individual compounds may be present at levels below the method detection limit or the biologically effective concentration, the combined effect of chemical mixtures may result in toxicity. Bioanalytical tools are cell-based assays designed to detect early cellular triggers that are prerequisites for, but may or may not result in adverse effects. The response measured in a cell-based assay for a given mode of toxic action (MOA; e.g., genotoxicity) reflects the total effect caused by all chemicals exhibiting that particular MOA. Bioanalytical tools thus have the ability to capture mixture effects, although causal relationships for single chemicals cannot be elucidated. Bioassays are selective, however, in that similarly structured chemicals tend to act via similar toxicological mechanisms leading to expression of similar MOAs. A given bioassay thus characterises the response of a particular compound group(s) that may be useful to establish source (e.g., photosynthesis inhibition by herbicides). The endpoints detected in bioassays can be grouped into non-specific (cytotoxic), specific (e.g., estrogenic, neurotoxic) and reactive (carcinogenic, mutagenic) MOAs. A comprehensive assessment will include a battery of bioassays covering a range of MOAs from all three main groups, preferably targeting non-specific toxicity, genotoxicity, mutagenicity and several different specific endpoints. Bioassays cannot replace chemical analysis but are valuable complementary tools for integrative water quality assessment and can be used as a screening tier to prioritise non-target analysis for toxicological relevance. This poster reviews the application of bioanalytical tools in water quality assessment to date, with recommendations for the future.

**TP031 DNA Reaction Rates and Relative Interaction Potency as a Tool for Rapid Screening Environmental Contaminants for Hazard using** Y. Feng, J. Gavina, Health Canada.

DNA damage represents a potential biomarker for determining the exposure risk to chemicals and may provide early warning data for identifying chemical hazards to human health. Here, we have demonstrated a simple chromatography-based method that can be used to rapidly screen for the presence of chemical hazards as well as to determine parameters relevant to hazard assessment. In this proof-of-principle study, a simple *in-vitro* system was used to determine the interaction of pollutants and probable carcinogens which include phenyl glycidyl ether (PGE), tetrachlorohydroquinone (Cl<sub>4</sub>HQ), methylmethane sulfonate (MMS), styrene-7,8-oxide (SO), and benzo[a]pyrene-7,8-dihydrodiol-9,10-epoxide (BPDE), a metabolite of benzo[a]pyrene (B[a]P), and 20 dyes, with single- and double-stranded DNA probes. Differences in potency were studied for chemical and DNA type. A relative interaction potency equivalency (PEQ) of a chemical was determined by ratio of interaction potency of a chemical to BPDE as the reference chemical in the reaction with single- and double-stranded oligodeoxynucleotides. PEQs were found to be BPDE > PGE > SO > MMS > Cl<sub>4</sub>HQ > other dyes for single-stranded oligodeoxynucleotides while they were found to be BPDE > PGE > Cl<sub>4</sub>HQ > MMS > SO > Toluidine Red > Tetraphenylethane glycidyl ether > other dyes for double-stranded oligodeoxynucleotides. Kinetics evaluation has been investigate for those positive interactions and revealed that BPDE reacted with both DNA probes at a significantly faster rate, as compared to the remaining test chemicals. Equilibrium was reached within an hour for BPDE, but required a minimum of 48 h for the remaining chemicals. The remaining chemicals possessed a relative kinetic order for reaction with DNA of BPDE >> MMS > SO > PGE > Cl<sub>4</sub>HQ for ds-DNA and BPDE >> SO ≈ Cl<sub>4</sub>HQ ≈ MMS > PGE for ss-DNA. We further found that the reaction potency, defined by dose-response between chemical pollutants and DNA, depended on the form of DNA present for reaction. Noteworthy, we found that relative PEQ did not follow the same kinetic trends. However, our preliminary findings suggest that reaction kinetics, in combination with relative interaction potency, may be a significant parameter that can be used to evaluate the hazard level of environmental pollutants.

**TP032 The Nuclear Receptor HR96 is a Toxicant Sensor in Daphnia**

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Sciences. The *Daphnia pulex* genome project is providing new insight into how an aquatic arthropod may respond to environmental stressors, including toxicants. We cloned *Daphnia pulex* HR96 (hormone receptor 96), a nuclear receptor orthologous to fruitfly HR96, and the mammalian xenobiotic receptors, CAR and PXR. A GAL4-HR96 chimeric receptor containing the DNA binding domain from yeast GAL4 and the ligand binding domain of *Daphnia* HR96 was made so that activation of the ligand binding domain in turn activated the pG5luc reporter that contains five GAL4 response elements during transactivation assays. We hypothesized that *Daphnia* HR96 is a promiscuous receptor activated by xenobiotics and endobiotics similar to the constitutive androstane receptor (CAR) and the pregnane X-receptor (PXR). Transactivation assays performed with a GAL4-HR96 chimera demonstrate that HR96 is a promiscuous toxicant receptor activated by a diverse set of chemicals such as pesticides, hormones, pharmaceuticals, and fatty acids. Several environmental toxicants activate HR96 including estradiol, pyriproxyfen, chlorpyrifos, atrazine, and methane arsonate. Repression of HR96 activity by hormones and pharmaceuticals such as triclosan, androstanol, and fluoxetine was also observed. About 50% of the chemicals tested altered HR96 activity. Interestingly, unsaturated fatty acids were common activators or inhibitors of HR96 activity, indicating a link between diet and toxicant response. The omega-6 and omega-9 unsaturated fatty acids linoleic and oleic acid activated HR96, but the omega-3 unsaturated fatty acids alpha-linolenic acid and docosahexaenoic acid inhibited HR96, suggesting that these two distinct sets of lipids perform opposing roles in *Daphnia* physiology. This also provides a putative mechanism by which specific anthropogenic chemicals or the dietary ratio of specific unsaturated fats may affect the ability of an organism to respond to a toxic insult. In summary, HR96 is a promiscuous receptor activated by several endobiotics and anthropogenic toxicants, including pesticides and pharmaceuticals.

**TP033 Phytotoxicity tests for rapid preliminary screening of urban stormwater quality** J.H. Myers, M. Allinson, University of Melbourne / CAPIM; V.J. Pettigrove, The University of Melbourne / CAPIM; G. Allinson, Department of Primary Industries, Victoria / Future Farming Systems Research Division; G. Rose, P. Zhang, A. Bui, Department of Primary Industries / Future Farming Systems Research Division. In Australia the reuse of stormwater provides an alternate source of water for non-potable uses during our regular periodic severe water shortages. However, urban stormwaters contain variable contaminant profiles, which pose a challenge for monitoring and risk assessment. New cost-effective, rapid screening tools are required to provide assurance that recycled stormwater is fit for purpose. Microalgae constitute a major component of the non-target organisms at risk from herbicides and, like higher plants, play a fundamental role in trophic web and biogeochemical cycles. Similarities between the structure and function of microalgal communities and that of higher plants make microalgae ideal indicators of the potential effects that herbicides in stormwater may have on receiving stream floral communities or on higher plants if reused on our parks and gardens. In this pilot study over a period of 6 months, 5 sites receiving stormwater from small catchments with different urban land-use were assessed for toxicity using a 72hr phytotoxicity bioassay, while herbicide profiles were determined using LC-MS/MS. The phytotoxicity bioassay highlighted significant toxicity during the 6 month study although the response was variable between sites. For example, at site 34 (rural site influenced by airport runoff), 10 to 88% inhibition relative to controls was observed 5 out of 6 months. At site 204 (industrial), toxicity was observed during the first 2 months (10-43% inhibition), but not observed thereafter. At sites 202 and 316 (urban residential with some industrial activities), toxicity was only observed during 1 month of the study (65% and 39% inhibition respectively), while at site 205 (residential with a shopping mall), highest toxicity was observed during the 6<sup>th</sup> month (16% inhibition). Of note was that during the 3<sup>rd</sup> month no toxicity was observed at any of the sites. Chemical analytical data indicated that simazine and 2,4-D were the dominant herbicides (100 and 93% of samples at concentrations ranging from the non detectable to 4.45 µg/L and 2.3 µg/L, respectively). Results from this study demonstrate the utilization of phytotoxicity tests in combination with chemical analyses as a valuable addition to test batteries for rapid preliminary screening in stormwater quality assessment.

**TP034 Toxicity of Environmental Compounds in Fish Cell Assays: A rapid monitoring tool for assessing contaminant effects** M. Brown-Augustine, University of California, Berkeley / Department of Nutritional Science and Toxicology; C. Clark, University of California, Berkeley /

Nutritional Sciences and Toxicology; L.E. Lee, Wilfrid Laurier University / Department of Biology; C. Vulpe, University of California, Berkeley / Nutritional Sciences and Toxicology. There is limited toxicological test data available for thousands of commercial chemicals that potentially pose adverse environmental effects. Current evaluations of toxicity rely on standardized whole-animal toxicity tests that are costly, time-consuming and provide little or no mechanistic insight. In addition, changes in the regulatory requirements of Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) require a need for alternatives to animal testing. New screening approaches, such as High Content Screening (HCS) assays, measure multiple cellular endpoints simultaneously and have renewed interest in cell-based toxicity assays. For example, the EPA and the NIEHS are evaluating cell-based assays for use in human toxicology. We are collaborating with the EPA to evaluate cell-based assays in fish for extrapolation to higher-level endpoints (LC<sub>50</sub>). We are in the process of screening 309 chemicals of ToxCast Phase I in rainbow trout and fathead minnow cell lines, which are representative of freshwater aquatic toxicity test species. Preliminary results indicate a measurable dose-response relationship (EC<sub>50</sub>) of fish toxicity with environmental contaminants.

**TP035 Gene Assembly Selection for Toxicity Assessment and Identification Using High-dimension Toxicogenomics Data** C. Gao, Northeastern University / Civil and Environmental Engineering; D. Weisman, University Of Massachusetts Boston / Department of Biology; A. GU, Northeastern University / Department of Civil & Env Engineering, Biotechnology Initiative Program. High-throughput screening techniques, such as whole reporter cell array, are useful toxicogenomic tools for toxicant detection and toxicological mechanism investigation. However, it remained an open question in the selection of a set of relevant genes that could be used as an accurate biomarker for specific chemicals or mechanisms. Current solution still relies heavily upon prior toxicological knowledge and expert experience. In addition, the advances of monitoring technology now allow for continuous measurement of the array signal to bring in more dynamic information, resulting in much higher data dimensionality. The high dimensional data require more complex analysis procedure because of the inherent correlation nature of time series for each gene, in addition to the multifarious relationships among genes. We present here an analysis framework including data mining and multivariate time series analysis techniques, which could be potentially widely used for gene assembly selection to ensure sufficient resolution yet with minimal redundancy. We first demonstrated the high-resolution power of the time series whole-cell array data for toxicant differentiation with high (90-95%) accuracy, using k-nearest neighbor algorithm in cross-validation test. We then compared and showed that certain metrics such as correlation coefficient work more properly for the purpose of classification, which would help further our understanding of potential relationship among dynamic gene expression. Thirdly, we employed feature selection, specifically backward selection algorithm to find a subset of genes that could maintain the prediction power with acceptable accuracy reduction but also with high biological relevance. The ascending biological relevance could be confirmed by the help prior knowledge using gene ontology analysis, which could provide the statistical significance of a set of genes share common toxicological functions/pathways. The other strategy in identifying gene subset we took was to incorporate feature transformation with time series analysis techniques, such as ARIMA model, wavelet analysis and hidden Markov model, to construct more representative and information dense variables and then to select more relevant gene set by conventional low-dimensional statistical analysis methods.

**TP036 Green Friedel-Crafts Reaction of Aromatic Compounds with Glyoxamides** N. Gathergood, Dublin City University / School of Chemical Sciences; E. Kowalska, H. Xie, Dublin City University / School of Chemical Sciences. The Friedel-Crafts reaction of aromatic compounds with carbonyl compounds is one of the fundamental reactions in organic chemistry. Numerous examples of addition reactions of aromatic compounds to activated carbonyl and α-dicarbonyl compounds, such as glyoxylate, trifluoropyruvate, and phenylglyoxal, in the presence of Lewis acids and organocatalysts are known. It provides a simple procedure for the preparation of optically active mandelic acid derivatives, which are important building blocks for many biologically active compounds. Herein, we present the catalytic Friedel-Crafts reaction of aromatic compounds including dimethylaniline to a series of glyoxamides catalyzed by various catalyst systems. We found that the reaction led to the formation of double addition products in the presence of



Lewis acid catalysts. Further study of the Friedel-Crafts reaction between *N*-methylpyrrole and glyoxamides in aqueous  $\text{NaHCO}_3$  demonstrated that the reaction selectively produced mono-addition products. Our work provides a 'greener' synthesis of bioactive  $\alpha$ -hydroxyamides and  $\alpha$ -hydroxyamino cores via green Friedel-Crafts type methodology. A detailed study including expanded reaction scope, catalyst optimization, solvent recycling and solvent effect will be presented.

**TP037 Green Chemistry Principles applied to the Synthesis of Novel Opiate Analogues** N. Gathergood, Dublin City University / School of Chemical Sciences; D. Canning, A. Coughlan, Dublin City University; D. Finn, National University of Ireland, Galway / Department of Pharmacology & Therapeutics. The constant pressure to prepare compounds in a more efficient manner has placed the process by which traditional synthetic chemistry is conducted under scrutiny. Pharmaceutical companies desire short, efficient and environmentally friendly synthetic methods. One way that offers this prospect is the minimization of the use of protecting groups in synthesis. A protection/deprotection strategy introduces at least two steps, incurring costs of reagent and waste disposal, and leads to a reduced overall yield. We herein wish to report our short two step synthetic approach with high yields which now leads to a diverse library of novel analgesic compounds. Binding data to the  $\mu$  opioid receptor is also presented.

**TP038 Bioderived ionic liquids for catalysis** N. Gathergood, Dublin City University / School of Chemical Sciences; S. Bouquillion, N. Ferlin, Université de Reims / Institut de Chimie Moléculaire de Reims; M. Ghavre, Dublin City University / School of Chemical Sciences. Ionic liquids (ILs) are products with a low melting point composed of an organic cation and an anion. Their properties (non-volatility, high capacity of solubilisation, thermal and chemical stability etc) make them good substitutes for usual organic solvents, and ILs are already used for several applications: electrochemistry, organic synthesis, catalysis, separation, and complexation. But recently, it was discovered that some common ILs are not very biodegradable, which is a disadvantage to consider them as "green solvents". Several works were carried out to improve the biodegradability of ILs by introducing hydrolysable functions on the cation and some of these compounds were used for selective hydrogenations of dienes. We propose that using renewable resources to form ILs could improve the biodegradability of the compounds. Our focus has been on the synthesis of ILs with a bio-derived anion, such as acids obtained from biomass (e.g. lactic acid, tartaric acid), sugar (osidic acid, D-xylose) or amino acids (proline). Their utilization in different catalytic reactions (hydrogenations, Pd catalyzed coupling reactions) was studied and their antimicrobial toxicity, ecotoxicity and biodegradation determined.

**TP039 Morphine – A sustainable platform molecule from biomass** N. Gathergood, Dublin City University / School of Chemical Sciences; S. Long, Dublin City University / School of Chemical Sciences. Enantiopure compounds are of great importance to the chemical industry especially as pharmaceuticals. One strategy in synthesizing enantiopure compounds involves the use of asymmetric catalysts such as organocatalysts. These catalysts are small chiral organic molecules used in substoichiometric amounts to promote organic reactions. However, for organocatalysts to be classified as green reagents, their biological activity, (eco)toxicity and biodegradation needs to be assessed. In addition for these organocatalysts to be sustainable, chiral building blocks readily available from Nature are preferred. Herein, we present results from our study using sustainable opiate alkaloids morphine and codeine as chiral building blocks for organocatalysts. Several new organocatalyst classes are disclosed, their effectiveness in asymmetric transformations, and our strategy to reduce undesirable biological activity associated with many opiate compounds.

**TP040 Safer Ionic Liquids which are fit for purpose – Biodegradability vs Stability – A Kobayashi Maru Scenario?** N. Gathergood, Dublin City University / School of Chemical Sciences; M. Gurbisz, Dublin City University / School of Chemical Sciences; C. Jungnickel, Gdansk University of Technology / Department of Chemistry. Biodegradation studies in our group have focussed on ionic liquids containing ester and amide groups. This work was inspired by the development of biodegradable surfactants over the last 50 years. We have reported several examples of readily biodegradable ionic liquids containing esters, and examples containing amides with low antimicrobial activity. There is a balance required between stability of the ionic liquid to the conditions of the application and ultimately

biodegradation. Our latest progress towards these goals includes a detailed stability (hydrolysis) study, stability in a range of metal catalysed reactions (inc. Hydrogenation), and as electrolytes in DSSC (Dye Sensitized Solar Cells) vs biodegradation.

**TP041 Analysis of Drugs of Abuse and Their Metabolites via On-line SPE-HPLC-MS/MS for Communal Assessment of Consumption** N. Heuett, Florida International University / Department of Chemistry and Biochemistry, Florida International University; P. Gardinali, Florida International University / Department of Chemistry and Biochemistry. Consumption of drugs of abuse is a growing concern affecting populations worldwide. Individual enforcement is usually a very intrusive process that requires voluntary forceful cooperation. Communal monitoring of drug use can easily be achieved by analyzing raw sewage from buildings, localized facilities or region-wide wastewater treatment plants. An on-line SPE-HPLC-MS/MS method was developed for the analysis of 16 drugs of abuse and their metabolites from the following families: Amphetamine-like compounds, opioids, cocaine, cannabinoids, and lysergics. The on-line SPE method was performed implementing an EQUAN technique, which involved the preconcentration of 5 mL filtered samples in a (20 X 2.1 mm) HyperSep Retain PEP loading column; followed by the separation of analytes in a Hypersil Gold PFP (50x2.1 mm) particle size (1.9 $\mu$ m) analytical column. A heated electrospray ionization source was employed in the positive mode, and a Thermo TSQ Quantum Access Triple Quadrupole mass spectrometer was used to monitor compounds by means of two SRM transitions for quantitation and confirmation purposes. On-line SPE recoveries ranged from 70-142 % for all the compounds, with the exception of  $\Delta$ -9-THC (13.5%). Good linearity ( $0.993 > r^2$ ) was shown in the range between 0 – 1000 ng/L for all compounds. Method precision was calculated as the relative standard deviation (RSD) of triplicate samples, yielding an average RSD of less than 13 %. Triplicate reclaimed water samples collected from Florida International University BBC campus suggested the presence of MDEA and EDDP metabolites at low concentrations of  $19.2 \pm 3.8$  ng/L and  $2.5 \pm 0.5$  ng/L respectively. A study of raw sewage collected from selected campus dorms with isolated wastewater systems was conducted to estimate the potential consumption of prescription or illegal drugs within in the student population. Cocaine and two of its metabolites (benzoylecgonine and cocaethylene) were found at concentrations of  $38.3 \pm 2.1$ ,  $45.2 \pm 0.9$ , and  $13.6 \pm 2.0$  ng/L respectively.

**TP042 Stability of Dioctyl Sulfosuccinate (DOSS) towards photodegradation and hydrolysis under simulated laboratory conditions** S. Batchu, FIU / Department of Chemistry and Biochemistry, Florida International University / Department of Chemistry and Biochemistry, FIU / graduate student; P.R. Gardinali, Florida International University / Department of Chemistry & Biochemistry and SERC. Corexit dispersants have been used to help mitigate the environmental impacts of oil spills. Corexit 9500A was used both at the surface and at depth during the response to the DWH incident. An anionic surfactant, Dioctyl sulfosuccinate sodium salt (DOSS) is one of the main components of Corexit 9500A. Besides biodegradation, abiotic transformation could also play an important role on the environmental stability of dispersant components. Photodegradation experiments were conducted using Rayonet photochemical reactors at two different wavelengths (254 nm and 320-380 nm). A SunTest, solar simulator equipped with a xenon lamp producing constant spectrum of light (300-800nm) was also used as a surrogate of natural sunlight. Degradation kinetics was studied in two matrices reagent water (RW) and salt water (SW). Stock solutions of 100  $\mu$ g/L DOSS were placed in 30-mL quartz tubes. Dark samples and blanks were used as positive and negative controls. 1-mL samples of the test solutions were collected into brown glass vials amended with 16  $\mu$ L of DOSS-13C4 (2 mg/L), and 484  $\mu$ L of acetonitrile. Samples were analyzed by direct injection LC-MS/MS for the target compounds and transformation products (TPs). DOSS degradation followed first order kinetics. Degradation at the most energetic wavelength (254nm) was slow with a half-life of 0.6 h for RW and 3.4 h for SW. As expected, degradation was much slower at 350nm with half-lives of 17 and 16 days for RW and SW respectively. A single TP resulting from the cleavage of the octyl- chain was identified from the experiments at 254 nm. LC/MS-MS analysis (Q/Q), followed by HRMS confirmation (Q-TOF) showed a molecular ion with  $m/z$  309.1014 with a corresponding formula of  $\text{C}_{12}\text{H}_{21}\text{O}_7\text{S}$  ( $+0.32$ ppm) and fragmentation pattern consistent with des-octyl-DOSS. TP309 was also observed during base hydrolysis.

**TP043 Photodegradation of Sucralose under simulated solar radiation: implications for its long-term environmental stability** S. Batchu, FIU / Department of Chemistry and Biochemistry, Florida International University / Department of Chemistry and Biochemistry, FIU / graduate student; P.R. Gardinali, Florida International University / Department of Chemistry & Biochemistry and SERC. Sucralose (Splenda) is a chlorinated derivative of the disaccharide sucrose in which three hydroxyl groups are replaced by chlorine atoms. Sucralose is used as an artificial sweetener in about 4000 different products worldwide. Even though human consumption of sucralose has been found safe it is considered as an emerging contaminant due to its extraordinary persistence in the environment. The input of sucralose to the environment is likely through municipal wastewater treatment effluents. In recent years sucralose occurrence has been increasingly reported in various surface waters in concentration that easily reach a few µg/L. As many polar and highly soluble organic compounds its environmental fate is mostly influenced by biodegradation, partition and photo degradation. Previous studies determined that sucralose is very resistant to biodegradation and has minimal affinity for environmental surfaces. This study investigates the effects of photolysis as a way to understand the potential persistence of sucralose in aquatic ecosystems. Photolysis was studied under three different light sources i.e. UV 254nm, UV 350 nm and a SunTest solar simulator. Three matrices, reagent water (RW), canal water (CW) and salt water (SW), were chosen to study the influence of matrix on the rate of photolysis. The experimental solution was prepared at 250 µg/L of sucralose in all matrices and transferred to 3-30mL quartz tubes. One tube filled with 30 ml of RW acts as blank. One tube was completely covered with aluminum foil to prevent light exposure and used as dark control and the third tube is exposed to light. At specified time intervals, 380 µl of aliquots were collected into amber glass vials to which 20 µL of sucralose-d6 (10 mg/L), was added and analyzed by LC-MS/MS with no further processing. Decay of sucralose at UV 254nm is a first order rate reaction with a half-life of 5.4 and 13 h in RW and CW, respectively. No degradation was seen in SW within 15 days at UV 254nm. In 350nm light source, sucralose degraded with a half-life of 48 days in RW and there is no appreciable degradation (< 5%) in CW or SW within 30 days. In the absence of biodegradation, sucralose will likely persist in surface or treated waters even after UV irradiation.

**TP044 Contaminants of Emerging Concern in Source and Treated Drinking Waters from 25 Drinking Water Treatment Plants: Perfluorinated Compounds** S. Boone, U.S. EPA / Office of Chemical Safety and Pollution Prevention, USEPA/ECB / USEPA/OCSP/OPP/BEAD/Environmental Chemistry Laboratory; T. Boone, C. Byrne, C. Vigo, USEPA/OCSP/OPP/BEAD/Environmental Chemistry Laboratory; B. Guan, A2R; S.T. Glassmeyer, U.S. EPA / Office of Research and Development, U.S. EPA / NERL/MCEARD/CERB; E.T. Furlong, U.S. Geological Survey / National Water Quality Laboratory; D. Kolpin, USGS; J. Ferrario, USEPA/OCSP/OPP/BEAD/Environmental Chemistry Laboratory. Contaminants of emerging concern (CECs) are being found at trace levels (ppt) in domestic drinking water. The USEPA and USGS conducted a study sampling source and treated water from 25 drinking water treatment facilities in the US for the detection of over 200 emerging chemical and microbiological contaminants. The source waters from these facilities included ground wells, reservoirs, and rivers. The contaminants of interest included: prescription and nonprescription pharmaceuticals and their metabolites, perfluorinated compounds, industrial chemicals, fragrances, polycyclic aromatic hydrocarbons, hormones, pesticides, detergent related chemicals, plant and animal sterols, phosphorus based flame retardants, nanomaterial, bacteria, fungi, protozoa, and viruses. Seventeen perfluorinated compounds (PFCs) were analyzed in both source and treated water by the current method developed by the Environmental Chemistry Branch using LC/MS/MS detection. The most abundant PFCs of all the sites on average were: PFPeA > PFOA > PFBA > PFHpA > PFHxA > PFOS > PFHxS > PFNA=PFDA=PFBS > PFUnDA > PFDODA. The most prevalent PFCs based upon detection frequency were: PFHxA > PFBS > PFBA = PFPeA = PFHpA > PFNA > PFHxS > PFOS > PFOA > PFDA > PFUnDA > PFDODA. Five PFCs were not detected at all: PFTtDA, PFTeDA, PFHxDA, PFOcDA, PFDS. The highest level of detects (ng/L: ppt) were: PFPeA@510, PFHpA@180, PFOA@110, PFBA@100, PFHxA@60, PFOS@50, PFHxS@50, PFNA@40, PFDA@30. The USEPA provisional health advisory for PFOA is 400 ppt and 200 ppt for PFOS. The concentrations in treated waters were similar to their source waters at all sites tested but one. At that one site, granulated activated carbon (GAC) was used and the PFCs were reduced:

PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFHxS, and PFOS(>96%), PFHxA(75%), PFPeA(40%), and PFBA(20%). Another site in the study also used GAC, but did not have the reductions. The discrepancy between the two facilities may depend upon degree of activation, and bed volume based upon flow rate. This study was designed to provide important baseline information on the CECs which persisted through drinking water treatments and was not designed to gather information representative of the drinking waters throughout the US. In most cases, the levels of PFCs were not affected by the drinking water treatment processes.

**TP045 Disposition of per- and polyfluoroalkyl substances during waste water treatment** J.P. Benskin, AXYS Analytical Services Ltd; M.G. Ikonomou, Institute of Ocean Sciences; S. Chotwanwirach, University of British Columbia; X. Liao, Institute of Ocean Sciences; L. Li, University of British Columbia / Civil Engineering; J.R. Grace, University of British Columbia / Department of Chemical and Biological Engineering; M.B. Woudneh, AXYS Analytical Services Ltd / R&D Chemist; J.R. Cosgrove, AXYS Analytical Services Ltd. The environmental occurrence of per-fluoroalkyl acids (PFAs) can arise from their direct use and also from the transformation of precursors (i.e. PFA-precursors). The relative importance of PFA-precursors as determinants of environmental PFA concentrations, along with the mechanism(s) under which precursor degradation occurs, is unclear. In this work we examined the disposition and time trends of PFAs and PFA-precursors during four steps of wastewater treatment. Samples of influent, effluent, biosolids and activated sludge collected from a waste water treatment plant over 8 months were analyzed for a suite of PFAs (C4-C14 perfluoroalkyl carboxylates, C4-C10 perfluoroalkyl sulfonates, and perfluoroalkyl phosphonates/phosphinates) and PFA-precursors ((N-alkyl substituted) perfluorooctane sulfonamides, fluorotelomer acids, and mono and di-substituted polyfluoroalkyl phosphate esters). This is the first study to comprehensively investigate the fate and behavior of commercial per- and polyfluoroalkyl substances and their degradation products during wastewater treatment. These data provide useful information for the development of capture, containment, and remediation strategies for per- and polyfluoroalkyl substances.

**TP046 Monitoring Triclosan and Triclocarban Using Active and Passive Sampling Techniques in Fountain Creek and Arkansas River, CO** P. Gautam, Colorado State University-Pueblo / Chemistry; J.S. Carsella, Colorado State University-Pueblo / Department of Chemistry; C.A. Kinney, Eastern Washington University / Chemistry & Biochemistry, Colorado State University-Pueblo / Chemistry. The presence of antimicrobials Triclosan (TCS) and Triclocarban (TCC) in Fountain Creek, CO, a wastewater dominated stream, and Arkansas River, CO was measured using both active and passive integrative sampling techniques. Additionally, data from this study was used to determine the primary mechanism of transport, dissolved vs suspended sediment, of TCS and TCC within this surface water system and the impact of Fountain Creek, a tributary of the Arkansas River, on the presence of TCS and TCC in the Arkansas River. Five different sampling sites in the Fountain Creek Watershed and Arkansas River were selected for this study. To date the quantity of TCS and TCC in water using active sampling (water grab sampling & bedsediment sampling) and passive sampling (Polar Organic Chemical Integrated Sampler (POCIS) & suspended sediments sampler-PVC pipe) in spring flow condition has been measured. The concentration of TCS and TCC in water grab samples ranged from 2.8 ng/L to 10.8 ng/L and 0.9 ng/L to 5.9 ng/L, respectively. The transport of TCS and TCC via suspended sediment associated with surface water ranged from 0.02 ng/L to 0.46 ng/L and 0.02 ng/L to 0.72 ng/L, respectively. The concentration of TCS and TCC using passive sampling (POCIS) technique ranged from 3.85 ng/L to 78.78 ng/L and 0 ng/L to 1956.22 ng/L, respectively. Based on the analysis of these samples from all five sites, the quantity of TCC and TCS transported in the dissolved phase is substantially greater than that associated with movement of suspended sediment. Therefore, the primary transport mechanism of TCS and TCC (> 99%) is movement of surface water. Both TCS and TCC were below detectable concentrations in the inorganic-rich bulk bed sediments at any of the sample sites. By accounting for the average flow during the sampling period in Fountain Creek and Arkansas River just prior to their confluence, the impact of Fountain Creek on the quantity of TCS and TCC in the Arkansas River can be estimated. At their confluence, Fountain Creek discharges 53.64 mg/s TCS and 464.37 mg/s TCC into the Arkansas River adding to the 12.66 mg/s TCS and 46.51 mg/s TCC in the Arkansas River before the confluence.



Based on the time weighted average concentration of TCC and TCS in the dissolved and suspended sediment phases of Fountain Creek and Arkansas River at these sampling sites, Fountain Creek contributes about 91% and 81% of the TCC and TCS, respectively, that is transported below the confluence of these surface waters. A similar set of samples and measurements will be made during base flow conditions in late summer 2012.

**TP047 Monitoring Chemical Contamination in the Coastal Waters of the Caribbean using Passive Samplers – The Barbados Experience** L. Sandy

University of West Indies; E. Smith, University of West Indies / Department of Biological and Chemical Sciences. Despite the importance of the marine environment of the Caribbean, this vital resource is subjected to the direct or indirect influx of a suite of contaminants from a myriad of sources. Monitoring the levels of these contaminants and resultant impacts has always been a challenge for our region. Major hurdles to incorporating adequate and efficient analyses for chemical and pharmaceutical compounds are, the range of contaminants entering the sea, cost, time, laboratory capability and capacity, available technical expertise, coupled with the complexity and low concentrations associated with the saltwater matrix, and the ability to monitor contaminants on a continuous temporal basis. Monitoring the levels of pharmaceutical and chemical contaminants using Semi-Permeable Membrane Devices (SPMDs) and Polar Organic Chemical Integrative Samplers (POCIS) was demonstrated along the west coast of Barbados by the Faculty of Pure and Applied Science in collaboration with Trent University (Canada) and the Caribbean EcoHealth Programme. During 2011, SPMDs and POCIS were deployed at 9 sites between Holetown and Worthing at a water depth ranging from 25-60 feet and retrieved for analysis after 30 days in situ, a total of 3 times per site over the monitoring period, with each in triplicate. The results obtained indicate that SPMDs and POCIS are advantageous for monitoring contamination in the marine environment of Small Island States by: 1) allowing the rapid concentration and detection of a wide range of compounds from the saltwater matrix (as evidenced by laboratory based studies); 2) allowing for relatively easy quantification of the level of contaminants; 3) providing data and information for risk assessment of toxicity (as well as extracts for direct assessment); 4) assessment of a new group of emerging contaminants (information on such are lacking in the region); and 5) determination of the sources of the chemical and pharmaceutical contaminants (by the use of 'indicator' compounds). A total of 21 fungicidal compounds and 7 pharmaceutical and personal care products (PPCPs) were detected by initial analyses, which have implications for aquatic biota and are thought to contribute to the degradation of the marine environment. It is hoped to link this analysis with that of microbial resistance to evaluate the potential issues of antibiotics in the marine environment in the area, as well as the assessment of coral disease to the same end.

**TP048 Monitoring DEET in Water: Potential Interference from Solvents During LC-MS/MS Analysis** S. Merel

The University of Arizona / Chemical and Environmental Engineering; A. Nikiforov, Toxicology Regulatory Services; S. Snyder, The University of Arizona / Chemical and Environmental Engineering. The tremendous improvement of analytical capabilities over the last decade has made it relatively easy to measure trace levels of organic contaminants (TORCs) in a wide range of matrices. Consequently, emerging contaminants are now frequently monitored in the environment, particularly in wastewater and receiving water. Among TORCs, the insect repellent DEET has been frequently detected in US waters. DEET is even considered as a relevant indicator to assess the contribution of wastewater effluent in the receiving water. Recent papers have monitored DEET through sample concentration by solid phase extraction followed by liquid chromatography with tandem mass spectrometry (LC-MS/MS) analysis. While LC-MS/MS provides relatively high specificity, the concentration of DEET reported can reach several hundred ng/L, which seems unusually high for a chemical used only during the mosquito season. In addition, DEET often has been detected in laboratory blanks. Therefore, in this study we search for a potential interference from the mobile phase itself. Two mobile phase (water/methanol and water/acetonitrile) and two brands of organic solvents were tested on three different columns. The mobile phase was allowed to flow through the columns at 5% organic for 0; 5; 10; 20; 30 and 60 minutes before performing the analysis for DEET on a 0 µL injection (no sample injected and no contact between the needle and the vial). The results showed the signal of DEET (4 transitions monitored) increases with the flow time, which sustains the postulate of an interference in the mobile phase. To further confirm this hypothesis, HPLC water, methanol

and acetonitrile (two brands of each) were concentrated through evaporation below nitrogen stream and then analyzed for DEET by LC-MS/MS. All the concentrated samples exhibited a higher signal for DEET. In addition, the relative abundances of the four transitions monitored were similar to those observed when analyzing the analytical standard of the insect repellent. Such findings suggest a potential interference originated from the solvents during the analysis of DEET, which might lead to an overestimation of the actual concentration of this compound in the environment. Further research will be conducted to characterize this interference and determine to what extent it could influence the concentrations of DEET reported.

**TP049 Monitoring DEET in Water: Fundamental Study to Evaluate the Plausibility of Mimics** S. Merel

The University of Arizona / Chemical and Environmental Engineering; A. Nikiforov, Toxicology Regulatory Services; S. Snyder, The University of Arizona / Chemical and Environmental Engineering. The recent advances in analytical chemistry allow trace levels (ng/L or lower) of organic chemicals (TORCs) to be easily detected in environmental matrices. Consequently, punctual and routine analysis of wastewater and receiving water reveal the occurrence of more and more contaminants, most of them being classified under the term Contaminants of Emerging Concern (CECs). Among these CECs, the insect repellent DEET is one of the most frequently reported. However, the concentrations reported in several studies are relatively high (reaching hundreds ng/L) and not really consistent with the usage of DEET, which is expected to be limited to the mosquito season and localized to humid areas affected by mosquitoes. A potential explanation for these unexpected values could be the occurrence of one or more chemical mimics in the environment that would lead to an overestimation of DEET concentration in water. Therefore, we performed a fundamental study to evaluate the plausibility of such a hypothesis utilizing both GC-MS and LC-MS methods. A mimic would have the same retention time and transitions as DEET, which implies similar structure and mass. Based on these criteria, the compounds most readily available were researched through the websites of common chemical suppliers. Six potential mimics were identified for this study and analyzed through LC-MS, LC-MS/MS, GC-MS and GC-MS/MS. While 4 of the 6 potential mimics could be easily discarded because of an inappropriate retention time, the other 2 could not be resolved from DEET by LC-MS analysis. In addition, LC-MS/MS analysis showed similar transitions between these two compounds and DEET. Even though the relative abundances of each transition might be slightly different, detecting an anomaly or not only depends on the operator of the instrument and the level of tolerance accepted in each laboratory. In conclusion, the present study does not confirm a specific DEET mimic in the environment but clearly demonstrates the plausibility of such a hypothesis. Future work will extend the research on potential mimics to other compounds structurally similar to DEET and confirm whether or not the tangible mimics identified in the laboratory could actually occur in environmental samples.

**TP050 Degradation and Volatilization of Octamethyltrisiloxane (L3) in Soils** S. Xu

Dow Corning Corporation / Health and Environmental Sciences, Dow Corning Corp. / Health and Environmental Sciences; K. Doede, Dow Corning Corp.; C.A. Staples, Assessment Technologies, Inc.. Octamethyltrisiloxane (L3) (CAS No. 107-51-7) is a low-molecular-weight volatile methylsiloxane fluid primarily found as an impurity in many silicone products. The objective of this study was to determine the degradation and volatilization rates of L3 in soil. A loamy (Londo) soil from Michigan and a clayey soil from UK were first air-dried and then pre-conditioned in desiccators with the air at constant moisture levels: 32 %, 42%, 92% and 100% RH. The pre-conditioned soil samples were then spiked with <sup>14</sup>C-labeled L3 (in pentane) and incubated at three different temperatures. At each time interval, two samples were extracted sequentially with organic solvent and acidified water. The non-extractable radioactive residue in soil was determined by combustion using a biological oxidizer to check the mass balance of the spiked radioactivity. In closed systems, the recovery rates of the spiked L3 radioactivity under various moisture levels (including 100% RH) ranged from 93.6 % to 106.3 % with an overall average of 99.9 %. The degradation of L3 was more rapid as soil became drier with the half-lives of L3 at 22.5 °C being 1.5, 3.6, 6.2 and 120 days in Londo soil at 32% RH, 42% RH, 92% RH and 100% RH, respectively. Based on the profile of the degradation intermediates and final products, hydrolysis was the predominant degradation mechanism with activation energy of 63.5 kJ mol<sup>-1</sup>, similar to that for L3 hydrolysis in water. In open systems, the volatilization



of L3 was not significant at 32% RH due to strong sorption by dry clays. At 100% RH, however, the volatilization was the predominant process for removal of L3 from soil in the open systems, with a volatilization half-life less than 1 day, much faster than the degradation of L3 at the same moisture level in the closed systems. (This project was sponsored by The Silicones Environmental, Health and Safety Council of North America).

**TP051 Improving the Estimation of Physical Properties of Organosilicon Compounds** R. Boethling, US Environmental Protection Agency / Office of Pollution Prevention and Toxics; G. Kozerski, Dow Corning Corporation; W. Meylan, P. Howard, SRC Inc.. Reliable models for estimating physical-chemical properties of chemicals are critical for filling data gaps. Using new experimental data from industry-sponsored studies and the literature, combined with data from the SRC Environmental Fate Database and literature sources, several models in the US EPA's EPI Suite™ program were retrained for structures containing Si. The majority of the new partition coefficient and solubility data were for siloxanes, silanols, and silanes. In the case of the octanol-water ( $K_{OW}$ ) and air-water ( $K_{AW}$ ) partition coefficient, the revised models showed a 3- to 6-fold decrease in mean absolute error for the training sets (log basis), with the majority of this improvement associated with methylsiloxanes. For boiling point, approximately 20% of the training compounds were affected by retraining and showed a 10-fold decrease in standard deviation. Several models that depend on property estimates from revised models (for example, water solubility is estimated from log  $K_{OW}$ ) also benefited. Additional data were then collected from US new chemical (Premanufacture Notice; PMN) submissions and the open literature, and this structurally diverse set of organosilicon chemicals was used to independently validate the revised models. Results for Si-containing chemicals with measured boiling point, melting point, vapor pressure or  $K_{OW}$  confirm that the retrained models for these endpoints are more accurate. For example, for 126 organosilicon chemicals with measured boiling point that were notified as PMNs from 2002-2011, 27 (21%) were affected by retraining, and for these the mean absolute error decreased from 41 to 21 deg. Last, results from the revised EPI models and SPARC Performs Automated Reasoning in Chemistry (SPARC) are compared using the independent validation sets for boiling point,  $K_{OW}$  and vapor pressure.

**TP052 Using QSPRs, Chemical Fate Models, and the Chemical Partitioning Space to Investigate the Fate of Chemical Mixtures with High Number of Components** A.B. Gawor, University of Toronto / Departments of Chemistry and Physical and Environmental Sciences; F. Wania, University of Toronto Scarborough / Dept. of Physical & Enviro. Science, University of Toronto at Scarborough / Dept. of Physical & Enviro. Science. A number of substances are mixtures of very large number of constituents which vary widely in their properties, and thus also in terms of their environmental fate behaviour and the risk that they may pose to humans and the environment. Examples include: industrially used substances such as the chlorinated paraffins, technical pesticides such as toxaphene, and unintended combustion side products, such as mixed halogenated dibenzo-p-dioxins and dibenzofurans. Those substances are often mixtures of many thousands of individual compounds. Here we propose a simple methodology based on property estimation techniques and chemical partitioning space maps that could precede a more detailed chemical risk assessment for such substances. Through simple graphical superposition, the methodology seeks to address questions such as: Which constituents are likely to pose the greatest risk? Can individual constituents of those mixtures serve to represent the whole in such an assessment? How many different constituents would need to be assessed and how should they be selected? We illustrate our approach based on three sets of chemical substances.

**TP053 QSPR Study of the Biodegradability of Fragrance Materials** E. Papa, S. Kovarich, L. Ceriani, S. Cassani, University of Insubria; R. Boethling, US Environmental Protection Agency / Office of Pollution Prevention and Toxics; P. Gramatica, University of Insubria. Fragrance materials are structurally heterogeneous compounds that are widely used as ingredients in many consumer and personal care products. Their environmental occurrence, mainly in air and water compartments, is of potential concern for both humans and wildlife. Information on biodegradability is needed to assess risk from human and environmental exposure to these substances. Predictive approaches, such as quantitative structure-activity (property) relationships (QSA(P)R), can be used to predict biodegradability of untested substances in order to characterize their environmental persistence. In this

study QSPR classification models were developed for predicting ready biodegradability, based on measured data for a heterogeneous set of 196 chemicals. Over a thousand theoretical molecular descriptors were calculated by DRAGON and PaDEL software from energetically optimized 3D structures using the AM1 semi-empirical method. The structural information encoded in the molecular descriptors calculated for the studied dataset was first explored by multivariate analysis to identify possible outliers in the structural space. Two classification methods, Classification And Regression Tree (CART) and k-Nearest Neighbors (k-NN), were applied to perform the modeling. After initial random splitting of the dataset, and removal of seven structural and response outliers, the final models were developed on a training set of 145 chemicals, by selecting the best modeling variables (up to 4 descriptors) through Genetic Algorithm. The best QSPRs were validated on two external prediction sets of 44 and 51 chemicals, respectively. The structural applicability domain was verified by the leverage approach. Good values of sensitivity ( $S_n$ ) and specificity ( $S_p$ ) were calculated for the best models (ranges:  $S_n$  internal 0.74-0.97;  $S_n$  external 0.73-0.91;  $S_p$  internal 0.80-0.89;  $S_p$  external 0.77-0.86). In conclusion, the proposed QSPR models may be useful for screening fragrance chemicals even before they are synthesized. In this capacity they can be used to design fragrances that are less persistent than existing alternatives. Some of the proposed models are based on descriptors freely calculable online (PaDEL), which guarantees their accessibility and applicability.

**TP054 Investigation and Development of a Non-Invasive LC-MS/MS Quantitative Method to Determine the Stress Levels of Whales** K. Rosnack, A. Gledhill, J. Dunstan, Waters Corporation; A. Hall, P. Miller, Scottish Oceans Institute; C. Ramp, Mingan Island Cetacean Study. The conservation and management of large whale populations requires information about all aspects of their biology, life history and behavior. However, it is extremely difficult to determine many of the important life history parameters, such as reproductive status, without using lethal or invasive methods. As such, efforts are now focused on obtaining as much information as possible from the samples collected remotely, with a minimum of disturbance to the whales. Hormone analysis is of particular interest as high levels of progesterone can be used as an indicator of pregnancy status while other steroids such as glucocorticoids may be markers of the short term, acute stress response. The aim of this project is to determine if it is possible to detect the presence of hormones in whale blow samples, and see if the hormone levels could be related to stress due to their environmental situation. If successful the project would enable a non-invasive method to be developed to determine the stress levels of species that are difficult to study. Specific hormones, progesterone and cortisol, were monitored in samples of whale blow from a variety of different whale species. The results showed that both cortisol and progesterone were detected easily in the lowest calibration standard (0.5 pg/ $\mu$ L) suggesting lower detection limits are possible with further method development. Reproducibility was excellent, %RSD for both compounds were less than 2% for 5 replicates of a 5 pg/ $\mu$ L standard. Linearity for both compounds was excellent;  $r^2 = 0.999$  for cortisol and  $r^2 > 0.999$  for progesterone. Cortisol was detected in the majority (22 of 25) of samples at a level higher than the lowest calibration point. Progesterone was detected in 13 samples higher than the lowest calibration point. For all samples parallel collection of both MRM & full scan data was obtained and is shown for each of the species types. This set of data enabled other compounds to be simultaneously screened, and any matrix background to be monitored.

**TP055 An on-line SPE/LC-MS/MS method for the determination of multiple classes of antibiotics in environmental and reclaimed waters** V. Panditi, Florida International University / Dept of Chemistry and Biochemistry; P.R. Gardinali, Florida International University / Department of Chemistry & Biochemistry and SERC. An on-line preconcentration LC-MS/MS method was developed for the simultaneous analysis of 31 antibiotics (8 fluoroquinolones, 5 tetracyclines, 1 lincosamide, 7 macrolides, 9 sulfonamides and 1 penicillin) in various environmental water matrices i.e. reclaimed water, river water and seawater. A Thermo EQUAN™ system equipped with 6-port valve capable of switching between loading and analytical column was used for online preconcentration and analytical separation. Each sample (10 mL) was injected into a 10ml loop and transferred onto a loading column (Thermo Hypersep Retain-PEP 20 mm  $\times$  3.0 mm) at a flow rate of 2mL/min. The loading column was washed with 100% HPLC grade water for one minute at 2mL/min and the valve was switched to enable the load column to be back flushed onto the analytical

column (Thermo Hypersil gold 50 mm × 2.1 mm × 1.9 mm). A Thermo TSQ-Quantum Access QqQ MS equipped with H-ESI source (heated electrospray ionization) operated in positive ionization mode was used for the data acquisition. The total run time was 21 min. Confirmation of the presence of detected analytes was done using selective reaction monitoring. Two transitions were selected for each target analyte. Method detection limits of target compounds ranged from 0.5-14 ng/L, 6-29 ng/L in de-ionized water and reclaimed water, respectively. Using this method 55 reclaimed water samples were analyzed over a period of 12 months. Antibiotics detected most frequently in reclaimed water samples were sulfamethoxazole, ofloxacin, trimethoprim, nalidixic acid, erythromycin, clarithromycin, ciprofloxacin with concentrations ranging from 70.3-693 ng/L, 44.4-294 ng/L, 56.5-360 ng/L, 54.9-900 ng/L, 57.8-839 ng/L 14.1-577 ng/L and 6.9-143 ng/L respectively. River water samples (20) were collected from the Miami River that passes through urban areas of Miami known to have chronic water quality problems. Trimethoprim, doxycycline and nalidixic acid were detected in river water indicating that surface water is influenced by waste water intrusions.

**TP056 Determining Suspended Sediment Uptake of Micropollutants in Water Using Dynamic In-Situ Large Volume SPE Extraction** B. Hepner, Aqualytical Services Incorporated / Research & Development. Our fresh and marine waters are a complex and ever changing dynamic system. Simple snap shot sampling does not provide the necessary sample size in terms of volume and duration to meet pg/l or even ng/l detection levels that the emerging contaminants often require. One of the challenges associated with low level detection in water is that the concentration of free or dissolved micropollutants in the water will be greatly affected by TSS competitively adsorbing the hydrophobic analytes of concern. The traditional approach to the analysis of these micropollutants has been to use snapshot sampling, and pre-filtration to prevent clogging and interferences, followed by the use of high-resolution mass spectrometers. While this technique generally has very low detection limits, it is also very costly, and often provides only dissolved quantitation values. As evidence continues to mount regarding the bioaccumulative and toxic effects of C.E.C.s, there is a growing need for a sensitive, rugged, cost-effective method for these emerging compounds. To this end a submersible field SPE dynamic extraction device was developed. The device can simultaneously extract both the sediment and dissolved water fraction, using a two stage filter and SPE disk train. The extraction can represent up to 100 liters of water field extracted in-situ over a 36 hour time integrative duration, and will produce a quantitative solvent extract for both the sediment and dissolved analysis. The high volume of water extracted will allow laboratories to obtain high-resolution detection at a far lower cost using standard instrumentation. The results of a laboratory bench study will validate the ability of the device to extract large volumes of spiked water containing known sediment levels with different adsorptive capacity strengths, and differing Kow values of micropollutants. The validation study will use a novel mixed media extraction disk which can sequester both polar and non-polar targets in a single pass. The PBDE congeners, and the Phenoxy Herbicides, were chosen as the target analytes of this study, to illustrate the sediment uptake, and the ability to obtain ultra trace levels of these compounds in both a whole water and fractionated whole water/sediment comparison.

**TP057 Multi-residue determination of pharmaceutical and personal care products (PPCPs) in vegetables** X. Wu, J.L. Conkle, J. Gan, University of California, Riverside / Dept. of Environmental Sciences. Treated wastewater irrigation and biosolid amendment are increasingly practiced worldwide and contamination of plants, especially products that may be consumed raw by humans, by pharmaceutical and personal care products (PPCPs), is an emerging concern. A sensitive and reproducible method was developed for the concurrent measurement of 19 frequently occurring PPCPs in vegetables using ultra-performance liquid chromatography-electrospray ionization tandem mass spectrometry (UPLC-ESI-MS/MS) for detection, combined with ultrasonic extraction and solid phase extraction (SPE) cleanup for sample preparation. Deuterated standards were used as surrogates to account for matrix effects. The corrected recoveries ranged between 87.1 and 123.5% for iceberg lettuce, with intra- and inter-day variations less than 20%, and the method detection limits (MDLs) were in the range of 0.04 to 3.0 ng g<sup>-1</sup> dry weight (dw). The corrected recoveries were equally good when the method was used on celery, tomato, carrot, broccoli, bell pepper and spinach. The method was further applied to examine uptake of PPCPs by iceberg lettuce

and spinach grown in hydroponic solutions containing each PPCP at 500 ng L<sup>-1</sup>. Twelve PPCPs were detected in lettuce leaves with concentrations from 0.2 to 28.7 ng g<sup>-1</sup> dw, while 11 PPCPs were detected in spinach leaves at 0.04 to 34.0 ng g<sup>-1</sup> dw. Given the diverse chemical structures of PPCPs considered in this study, this method may be used for screening PPCP residues in vegetables and other plants impacted by treated wastewater or biosolids, and to estimate potential human exposure via dietary uptake of the produces.

**TP058 Analysis of Children's Products for Chemicals of High Concern to Children** A. Stone, Wa Department Of Ecology / Chemist; J. Grice, Washington Department of Ecology / Waste 2 Resources Program; H. Davies, Washington Department of Ecology / Waste 2 Resources, St of Washington / Dept of Ecology; J. Williams, Washington Department of Ecology / Waste 2 Resources. In 2008, the Washington Legislature passed the Children's Safe Product Act (Chapter 70,240 RCW) with the goal of identifying the use of toxic chemicals in children's products. The Children's Safe Product Act required Ecology to identify a list of chemicals of high concern to children (CHCCs) and gave specific directions on how the CHCCs were to be identified. CHCCs were defined as chemicals with known human health hazards and that had the potential of being exposed to children. Ecology prioritized the chemicals to an initial group with specific and well-established concerns. In 2011, the Washington Department of Ecology (Ecology) published a list of 66 CHCCs. This list contains a number of specific chemicals as well as several classes of chemicals. Using a phased-in approach and beginning in August 2012, manufacturers of children's products containing any of the 66 CHCCs must report that information to Ecology who will make the information available to the general public. In order to determine the level of compliance with the Children's Safe Product Act, Ecology initiated a children's product sampling program. The first products that must be reported to Ecology are those that are intended to be applied to the skin or placed in the mouth. Ecology visited several local Washington stores and purchased children's products that meet these requirements. These products were subjected to analysis for eight phthalate plasticizers, up to ten metals of concern, four paraben preservatives, formaldehyde (limited sampling), fifteen organic solvents or specific flame retardants. Only those product components that could potentially contain a chemical of concern were tested. In this poster, Ecology is reporting the initial results of this sampling effort and will document the types of products tested and the analytical results for the chemicals of concern. These results will provide an indication of whether any of the CHCCs remain in specific types of children's products and whether companies are in compliance with the Children's Safe Product Act. No attempt will be made to make any exposure assessment. The Children's Safe Product Act is based solely upon hazard and the potential for exposure.

**TP059 Comparison of ultrasound and QuEChERS methods for estrogens analysis in sediment** D. Bezerra, M. Marchi, São Paulo State University. Steroidal estrogens (estrone – E1, 17 β-estradiol – E2, estriol – E3 and 17 α-etylnylestradiol – EE2) have been studied about their occurrence in water bodies mainly in the liquid phase. However because their physicochemical properties (Kow and Koc) their presence in solid phase (sediment and/or particulate material) is expected and is very important to access the environmental risk of these substances. The available methods in the literature for estrogens analysis in solid matrices include usually a solid liquid extraction (ultrasound, Soxhlet, ASE, MAE) followed by an extract cleanup. On the other hand, the QuEChERS (quick, easy, cheap, effective, rugged, and safe) approach, early designed for pesticide analysis in fruits, proposes an efficient only one step method for sample treatment for organic compounds analysis. In this work we compare ultrasound with QuEChERS performance on estrogens analysis in river sediment. The estrogens analysis was carried out using HPLC-FLD. QuEChERS involves extraction with MeCN acidified partitioned from the sediment matrix (2.5g) using anhydrous MgSO<sub>4</sub> and NaCl followed by a dispersive-SPE cleanup with C<sub>18</sub> and primary secondary amine (PSA) or cartridge PSA 500mg. For ultrasound method, two and a half grams of dried sediment were subsequently extracted with MeOH, MeCN and acetone, ultrasonicated for 10 min each step, centrifuged and supernatant was collected. SPE cleanup was performed on an Strata-X cartridge. Extraction efficiency was defined as the ratio of the analyte peak area in the pre-spiked sample to that in the post-spiked sample. Fortification levels 2 μg g<sup>-1</sup> and 1 μg g<sup>-1</sup> for QuEChERS and ultrasound methods. The results of the average recoveries ranging for all compounds

from 60 to 84% (QuEChERS) and 65-104% (ultrasound), with coefficient variation (CV) values smaller than 20% in both methods. When using PSA cartridge for clean-up QuEChERS method showed highest recoveries (67-137%) and CV (>50%). The results has shown that these compounds can be detect in sediments by QuEChERS method combined dispersive-SPE, a technique greener than the others, it uses substantially smaller amounts of solvents and shortens extraction times with efficiency quite to traditional methods.

**TP060 Prediction of leachability of pharmaceuticals to groundwater by simulation models for groundwater contamination** T. Suzuki, Tokyo Metropolitan Institute of Public Health / Division of Water Quality, Tokyo Metropolitan Institute of Public Health / Department of Pharmaceutical and Environmental Sciences, Tokyo Metropolitan Institute of Public Health; Y. Kosugi, M. Hosaka, D. Nakae, Tokyo Metropolitan Institute of Public Health; T. Nishimura, Teikyo Heisei University. The present study was conducted to predict leachability of pharmaceuticals to groundwater using 3 simulation models for groundwater contamination such as GUS score, Jury's criteria, and Cohen's criteria, which were originally developed to predict leachability of pesticide to groundwater. The target pharmaceuticals were selected to be 3 non steroidal anti-inflammatory drugs (ibuprofen, diclofenac and mefenamic acid), 2 antibiotics (levofloxacin and clarithromycin), and a disinfectant (triclocarban), because they have been detected in compost samples of sewage treatment plants in Japan. A distribution coefficient (Kd) and an organic carbon normalized adsorption coefficient (Koc) of the pharmaceuticals were obtained with the batch equilibrium adsorption method according to OECD TG 106. A half-life of the pharmaceuticals in soils ( $T_{1/2}$ ) was measured according to OECD TG 307. As soil types, andosol and alluvial soil were selected to use, because they are representative agricultural soils in Tokyo. Koc values in andosol and alluvial soil of ibuprofen, diclofenac, mefenamic acid, clarithromycin and triclocarban were 563 and 1206, 602 and 1734, 298 and 1541, 1109 and 1027, and 10761 and 16055 cm<sup>3</sup>/g, respectively, while Koc value of levofloxacin was more than 90000 in both soils.  $T_{1/2}$  values in andosol and alluvial soil of ibuprofen, diclofenac and mefenamic acid were 11 and 13.6, 0.9 and 0.6, and 0.8 and 2.1 days, respectively, while  $T_{1/2}$  values of clarithromycin and triclocarban were more than 56 days in both soils. GUS scores of all pharmaceuticals were lower than 1.8. As for Jury's criteria, Koc values of all pharmaceuticals were higher than the value calculated by Jury's equation. In the case of Cohen's criteria, a pesticide is classified as a leacher, when its Koc is lower than 500 cm<sup>3</sup>/g, and its  $T_{1/2}$  is higher than 14 days. All 6 analyzed pharmaceuticals are thus predicted to be non-leachers. Field monitoring of these 6 pharmaceuticals in groundwater is necessary in order to confirm the currently obtained prediction.

**TP061 Development of Quantitative Structure-Property Relationship Models for Drinking Water Treatment Processes: Detoxification and Disinfection By-products** T.T. Sanan, US Environmental Protection Agency / NRMRL. With the proliferation of human-derived chemical contaminants in wastewater, a growing concern is the reactivity of these chemicals under drinking water treatment conditions. In some cases, drinking water treatment methods can remove threats by detoxifying contaminants through physical or chemical transformations; on the other hand, some treatment methods can generate harmful by-products. The diversity and rapid growth of synthetic molecules present in water supplies precludes an experimental assessment of the outcome of water treatment for all species, leading to interest in the development of predictive tools to estimate the propensity of molecules for both detoxification as well as formation of harmful by-products (DBPs) following drinking water treatment. To achieve this goal, quantitative structure-property relationship (QSPR) models have been developed to predict detoxification of potentially hazardous pesticides and herbicides (e.g. rates of reaction with hypochlorous acid) as well as for predicting the propensity of molecules to form several classes of DBPs, including *N*-nitrosamines. Computational methods employed include molecular mechanics, as well as both semi-empirical and density functional theory approaches to quantum chemical calculations.

**TP062 Background Concentration of PAHs and Dioxins/Furans in Gainesville Surface Soils: Exceedances of Florida's Allowable Risk Level** P. Anderson, Ohio EPA; J. Iannuzzi, A. Weaver, ARCADIS; M. Brouman, Beazer East, Inc.. Between 2009 and 2012 thirty-three background surface soils samples were collected from residential areas in Gainesville, Florida and

analyzed for polynuclear aromatic hydrocarbons (PAH) and dioxins and furans (D/F). Samples were collected from four distinct neighborhoods and also adjacent to busy streets. Concentrations varied between neighborhood and were generally higher along busy roads. Concentrations also appear to be higher in older neighborhoods. Florida has established a statutory allowable risk level of  $1 \times 10^{-6}$  as well as default residential soil cleanup target levels (SCTLs). The PAH concentration in approximately 30% of the background samples exceeded the SCTL, in some cases by more than 10-fold. The D/F concentration exceeded the SCTL in approximately 10% of background samples, and in one sample by more than 10-fold. Depending upon neighborhood, the average and upper tolerance limits of background PAH and D/F concentrations also exceeded the SCTLs. The results of this study also indicate that the "background risk," particularly of combined chemicals, can be much greater than Florida's allowable risk standard. The potential implications of this finding on cleanup of waste sites is discussed.

**TP063 Thermodynamic considerations of the sorption of aromatic amines** F.P. Donaldson, Rensselaer Polytechnic Institute / Department of Civil and Environmental Engineering; R. Ma, Rensselaer Polytechnic Institute / Department of Chemical and Biological Engineering; J. LaBombard, Rensselaer Polytechnic Institute / Department of Civil and Environmental Engineering; J. Shoulder, Rensselaer Polytechnic Institute / Department of Chemistry and Chemical Biology; M. Nyman, Rensselaer Polytechnic Institute / Department of Civil and Environmental Engineering. Hydrophobic organic compounds, including aromatic amines, pose a potential risk to humans due to their persistence and toxicity. Two such aromatic amines, aniline and benzidine, have been correlated with increased cancer risk in humans. The temperature dependent uptake of these compounds can yield useful insight into their sorption mechanisms. In the present study, 10-day sorption experiments for aniline, benzidine, and pyridine on a silty-clay soil were conducted at four temperatures (277, 281, 285, and 297K). Uptake followed the general trend of benzidine > aniline > pyridine under all experimental conditions. Nonlinear regression analyses were performed on each data set to determine both Freundlich and Langmuir isotherm parameter values. Freundlich N values remained constant for a given chemical over the temperature range measured, indicating that sorption site energies changed little over the 20 degree temperature range. Isothermic heats of adsorption were computed based on the Clausius-Clapeyron equation, combined with UNIFAC activity coefficients and Langmuir isotherm parameter values. Results showed that sorption of the chemicals were highly exothermic, with isothermic heats on the order of -13 to -96 kJ/mol. Methanol extractions were also performed under the assumption that the extract reflected reversibly bound (through cation exchange or hydrophobic partitioning) compounds. Chemical recovery was low at room temperature (average of 10% for aniline, 2% benzidine). Combined with the high heats of adsorption, these results imply that covalent binding was a contributing mechanism in the case of aniline and benzidine over the 10-day experiments.

**TP064 Parabens and their chlorinated derivatives in river waters** M. Terasaki, University of Shizuoka / Institute of Environmental Sciences; Y. Takemura, S. Nagashima, M. Makino, University of Shizuoka. Parabens are widely used as preservatives in pharmaceuticals and personal care products, and they can be easily chlorinated by chlorinated tap water. The resulting chlorinated derivatives might pose a higher potential risk to human and ecosystems than the corresponding parent parabens; however, the occurrence of such derivatives in rivers remains unknown. The present study was undertaken to determine 23 parabens and their chlorinated derivatives from rivers receiving effluents from sewage treatment plants in Shizuoka city, in the central Pacific region of Japan. The compounds were extracted by solid-phase extraction with a styrene polymer sorbent, trimethylsilyl-derivatized, and then identified by gas chromatography-mass spectrometry. Six chlorinated parabens and their primary degradation products, two chlorinated hydroxybenzoic acids, were found for the first time in river water. The analysis results also indicate that chlorinated derivatives preferentially partition to the suspended-solid phase in river water. With regard to the sources of chlorinated derivatives, chlorination tests with tap water revealed that the paraben was immediately chlorinated to yield the dichlorinated paraben. When an aqueous solution of the chlorinated paraben was irradiated with an ultraviolet light, chlorinated catechol was identified and revealed to be rather resistant to photochemical decomposition. This study offered new data on the existence forms of parabens and quantitative data on parabens in an aquatic environment.



**TP065 Stability and insulation ability of cationic and anionic surfactant foams sprayed on soils** S. Jeong, Kunsan National University / Dept of Environmental Engineering; J. Hur, Kunsan National University / Department of Environmental Engineering; J. Jeong, Kunsan National University / Department of Environmental Engineering. Surfactant foams have been used in the various fields in environmental remediation and industrial processes. Film stability and insulation ability of surfactant foams play significant roles in determining efficiency of surfactant foam use. Cationic surfactants seldom generate aqueous foams, while anionic surfactants easily generate stable foams. This study increased foam stability of cationic surfactants by using additives such as colloids and polyvinyl alcohols. Foam film stabilities of several cationic and anionic surfactant foams were evaluated in this study. This study also evaluated foam film stability and adiabatic ability of cationic and anionic surfactant foams sprayed on soils. Film stability and adiabatic capacity of surfactant foams were deeply related to the structure of chemicals and surface tension of surfactant solutions used.

**TP066 Endocrine Disrupting Pollutants in the Long Island Sound** J.A. Elmoznino, University of Connecticut / graduate student; P. Vlahos, University of Connecticut / Marine Science. Waste water treatment facilities (WWTF) input fresh water, nutrients, and contaminants to the Long Island Sound (LIS). While nutrient loads and concentrations for some pollutants are regulated, many emerging contaminants are not controlled as the full range of toxic effects are unknown, and the regulatory framework controlling their usage and allowable inputs into coastal waters are not established. The water and sediment of the LIS are routinely sampled for classical persistent organic pollutants; however, there are numerous emerging contaminants, with known endocrine disrupting and bioaccumulating properties that have yet to be detected in this economically important region. It is critically important to investigate inputs of emerging contaminants throughout the LIS to determine potential impacts on this estuarine ecosystem. Several WWTF that discharge into the LIS watershed were tested for known classes of endocrine-disrupting compounds (EDCs): perfluorinated compounds (PFC), phthalates esters (PAE), alkylphenol compounds (nonylphenol, octylphenol and bisphenol A) and steroidal estrogens. WWTFs are a known point source of these pollutants, and research indicates that biodegradation during secondary treatment may increase concentrations of some EDCs. The distribution of several target compounds between the suspended particulate and dissolved phases in the effluent waters was also determined.

**TP067 Comparative studies of mercury in lake ecosystems around major emitters in western Canada** M. Evans, Environment Canada; D. Muir, Environment Canada / Aquatic Ecosystem Protection Research Division, Environment Canada / Aquatic Contaminants Research Division; J.L. Kirk, Environment Canada / Research Scientist, Environment Canada / Aquatic Contaminants Research Division, Environment Canada; K. Bielefeld, Environment Canada / Water Science and Technology Directorate, University of Saskatchewan / Biology, University of Saskatchewan, National Hydrology Research Center; J. Keating, Environment Canada / Water Science and Technology Directorate. The impact of mercury emissions on lakes depends on several conditions including; the nature of the emission, precipitation rates; and limnological features, particularly including pH but also lake size (depth, surface) which affects mercury methylation rates. Here we present the results of our investigations of mercury in surface sediments and predatory fish in lakes around two major mercury emitters in western Canada. While the three coal-fired power plants in the Wabamun Lake area in Alberta had annual mercury emission rates of 387-732 kg over 2000-2010, mercury concentrations in surface sediments were low, averaging < 100 ng/g dry weight suggesting that only a fraction of the emitted mercury was deposited in the immediate vicinity of these power plants. Similarly, mercury concentrations in 500-mm northern pike (*Esox lucius*) were low ranging from 0.15-0.40 µg/g; low mercury concentrations may have been due to a combination of low mercury concentrations in sediments, alkaline waters, and high primary productivity which favored rapid growth rates of these fish. Mercury emissions were particularly high at Flin Flon, Manitoba, with rates of up to 19,900 kg a year until 1993; this has resulted in highly contaminated surface sediments in lakes in the vicinity of the smelter. Nevertheless, mercury concentrations were low in northern pike indicating that the most of the mercury deposited in the sediments was not bioavailable. Highest mercury concentrations were observed in lakes to the northwest where the slightly lower water pH may have favored mercury methylation. Although mercury emission rates have declined in recent years, mercury

concentrations in fish have not declined suggesting lags between decreases in mercury emissions and inputs from the watershed. Alternately other factors such as warming trends may be favoring the enhanced methylation of mercury.

**TP068 Application of a mercury residue dose-response model to predict fish injury at hazardous waste sites** N. Beckvar, NOAA/NOS / Office of Response & Restoration, NOAA / Office of Response & Restoration; T.M. Dillon, US Dept. of Commerce / NOAA, Assessment and Restoration Division, Dillon Environmental Consulting, LLC. Mercury is a contaminant of concern at many hazardous waste sites in the U. S. and globally. At aquatic sites, elevated mercury concentrations are measured in fish tissue, but direct impacts to the fish themselves often are not evaluated. We assessed impacts to fish at mercury-contaminated hazardous waste sites using a residue-based dose-response mercury toxicity model that estimates percent injury using endpoints related to mortality. The dose-response model was developed from laboratory toxicity studies and is a non-linear log versus normalized response model. A comprehensive mercury residue fish data set was compiled from mercury-contaminated hazardous waste sites with the goal of acquiring a complete range of mercury concentrations measured in field fish. Data were compiled from recent hazardous waste sites in the U. S. (45 species from 16 Superfund sites). Data from both recent and older U.S. and global sites (39 species) were also compiled in an attempt to capture the highest residues occurring in the environment. Whole-body mercury concentrations in fish at U.S. Superfund sites were generally below 1 mg/kg ww, but higher concentrations were measured at some locations and in the past. Model output (% injury) was compared to field observations of fish health to evaluate consistency between model output and effects in the field fish. The model predicted up to 79% injury in field fish. Important factors to consider when applying the model are presented and discussed.

**TP069 Toxicity of dietary methylmercury to fish: Derivation of ecologically meaningful threshold concentrations** D.C. Depew, Queen's University / School of Environmental Studies, Queen's University; N. Basu, University of Michigan / Department of Environmental Health Science; N. Burgess, Canadian Wildlife Services / Ecotoxicology and Wildlife Health Division; L.M. Campbell, Queen's University / School of Environmental Studies; E.W. Devlin, Hampden-Sydney College / Biology department; P.E. Drevnick, Institut National de la Recherche Scientifique; C.R. Hammer-schmidt, Wright State University / Department of Earth & Environmental Sciences; C.A. Murphy, Michigan State University / Department of Fisheries and Wildlife; M.B. Sandheinrich, J.G. Wiener, University of Wisconsin – La Crosse / River Studies Center. We determined threshold concentrations associated with adverse effects of dietary exposure to methylmercury (MeHg) from published results of laboratory studies on a variety of fish species. Adverse effects related to mortality were uncommon, whereas adverse effects related to growth occurred only at dietary MeHg concentrations exceeding 2.5 µg g<sup>-1</sup> wet weight. Adverse effects on behavior of fish had a wide range of effective dietary concentrations, but generally occurred above 0.5 µg g<sup>-1</sup> wet weight. In contrast, effects on reproduction and other sub-clinical endpoints occurred at dietary concentrations that were much lower (< 0.2 µg g<sup>-1</sup> wet wt). Although compelling evidence of comparable effects from field studies on fish or fish populations is scant, available data indicate that comparable adverse effects are nonetheless observed in wild fishes in environments corresponding to high and low MeHg contamination of food webs and are in general agreement with the threshold concentrations derived here from laboratory studies. These thresholds indicate that while differences in species sensitivity to MeHg exposure appear considerable, chronic dietary exposure to low concentrations of MeHg may have significant adverse effects on wild fish populations but remain little studied compared to mammals or birds.

**TP070 Modeling mercury concentrations in prey fish: Derivation of a common indicator of dietary exposure to fish and wildlife** D.C. Depew, Queen's University / School of Environmental Studies, Queen's University; N. Burgess, Canadian Wildlife Services / Ecotoxicology and Wildlife Health Division; L. Campbell, Queen's University / School of Environmental Studies. The National Descriptive Model for Mercury in Fish (NDMMF) developed by the USGS was applied to a Canada – wide dataset of fish mercury (Hg) concentrations in order to estimate Hg concentrations in a model prey fish to develop a common indicator of dietary methylmercury (MeHg) exposure to piscivorous fish and wildlife. Calibration of the model to a dataset of 231,063 observations yielded reasonable estimates of Hg in

fish. Hold-out validation indicated that the NDMMF provided relatively unbiased model coefficients and reasonable estimates of Hg in fish. In general, prediction error was greatest at sites with only a small number of observations and no strong spatial biases in model error were observed (with the exception of sites contaminated by point source Hg loading or recent impoundment) indicating that the derived indicator of dietary exposure ( $Hg_{PREY}$ ) provides a reasonably unbiased exposure indicator. Estimated Hg concentrations in prey fish ( $Hg_{PREY}$ ) between the years of 1990 – 2010 averaged  $0.09 \pm 0.07 \mu\text{g g}^{-1}$  across 1,936 locations nation-wide, and increased from west to east. The broad scale geographic pattern of  $Hg_{PREY}$  is consistent with independent measures of MeHg exposure in piscivorous wildlife and the general conceptual model that predicts increased Hg bioaccumulation in food webs of oligotrophic, acid sensitive aquatic ecosystems in forested catchments. Independent verification through a properly randomized sampling program offers the most rigorous approach to verify the extent and magnitude of the pattern of  $Hg_{PREY}$  observed here, but we suggest that the  $Hg_{PREY}$  dataset offers a unique standardized indicator to provide a first estimate of potential risks to piscivorous fish and wildlife at a previously unexamined scale.

**TP071 Screening benchmarks for dietary methylmercury (MeHg) exposure in the common loon (*Gavia immer*): Rationale for use in ecological risk assessment** D.C. Depew, Queen's University / School of Environmental Studies, Queens University; N. Basu, University of Michigan / Department of Environmental Health Science; N. Burgess, Canadian Wildlife Services / Ecotoxicology and Wildlife Health Division; L.M. Campbell, Queen's University / School of Environmental Studies; D.C. Evers, Biodiversity Research Institute; K.A. Grasmann, Calvin College / Department of Biology; A.M. Scheuhammer, Environment Canada / National Wildlife Research Centre. The current understanding of methylmercury (MeHg) toxicity to avian species has improved considerably in recent years and indicates that exposure to environmentally relevant concentrations of MeHg through the diet can adversely affect various aspects of avian health, reproduction and survival. Fish-eating birds are at particular risk for elevated MeHg exposure, so we surveyed the available primary and secondary literature to summarize the effects of dietary MeHg on the common loon (*Gavia immer*) and to derive ecologically relevant toxic thresholds for dietary exposure to MeHg in fish prey. After consideration of the available data, we propose three screening benchmarks of 0.1, 0.18 and  $0.4 \mu\text{g g}^{-1}$  wet weight MeHg in prey fish. The lowest benchmark ( $0.1 \mu\text{g g}^{-1}$  wet wt) is the threshold for adverse behavioral impacts in adult loons and is close to the empirically determined NOAEL (no observed adverse effect level) for sub-clinical effects observed in captive loon chicks. The remaining benchmarks (0.18 and  $0.4 \mu\text{g g}^{-1}$  wet wt) correspond to MeHg levels in prey fish associated with significant reproductive impairment and reproductive failure in wild adult loons. Overall, these benchmarks incorporate recent findings and reviews of MeHg toxicity in aquatic fish-eating birds, and provide the basis for a national ecological risk assessment for mercury and loons in Canada.

**TP072 In ovo exposure to methylmercury chloride is not associated with neurochemical or behavioral changes in white leghorn chicken hatchlings** J. Rutkiewicz, M. Bradley, K. Mittal, University of Michigan; N. Basu, University of Michigan / Department of Environmental Health Sciences, University of Michigan / Department of Environmental Health. Mercury (Hg) is a documented neurotoxicant that has been associated with alterations ranging from subclinical neurochemical changes to clinical brain lesions. Studies of birds in the field and laboratory have identified links between Hg exposure and changes in cognitive and motor skills. Ultimately, these behavioral changes may be related to impacts on survival or the reproductive impairments that have been observed in several studies. Avian embryos are exposed to maternally deposited methylmercury (MeHg), but few studies have addressed the potential neurochemical and behavioral effects of this developmental exposure on hatchlings. The objective of this work is to determine if *in ovo* exposure to methylmercury chloride (MeHg-Cl) is associated with neurochemical or behavioral changes in white leghorn chicken hatchlings. We injected white leghorn chicken eggs with MeHgCl (0, 3.2, 6.4 mg/g egg) at incubation day 0 and incubated until chicks hatched at day 21. We tested one day old and one week old chicks for righting response, startle response, and balance on level and angled balance beams, and euthanized chicks to measure Hg concentrations and NMDA and GABA receptor levels and glutamine synthetase (GS) and glutamic acid decarboxylase (GAD) enzyme activity in the brain. Brain (cerebrum) Hg

concentrations reached 4.0 mg/g (wet weight) in one day old chicks and 4.3 mg/g (wet weight) in one week old chicks. Despite this relatively high accumulation of Hg in the brain, we saw no relationships between dose group or brain Hg concentrations and neurochemical biomarkers or behaviors. These findings may indicate that chickens are relatively insensitive to Hg's neurological impacts or that embryonic exposure is insufficient to alter behavior and neurochemistry in young chicks.

**TP073 6:2 FTOH anaerobic biodegradation in digester sludge and titanium (III) citrate/VB12 facilitated reductive defluorination of 6:2 FTUCA** S. Zhang, Peking University / Environmental Science; P.K. McCausland, DuPont / Haskell Global Centers for Health and Environmental Sciences; B.W. Wolstenholme, B. Szostek, DuPont; X. Lu, Peking University / Environmental Science; N. Wang, DuPont, E.I. du Pont de Nemours & Company, DuPont; R.C. Buck, DuPont / Chemical and Fluoroproducts. PFASs [ $\text{F}(\text{CF}_2)_n\text{SO}_3^-$ ] and PFCAs [ $\text{F}(\text{CF}_2)_m\text{COO}^-$ ] are detected in the environment and biota due to their broad historical applications. Products containing long-chain PFASs and PFCAs or potential precursors are being phased out and replaced with shorter-chain alternatives based on perfluorobutane sulfonate and carboxylate, 6:2 FTOH [ $\text{F}(\text{CF}_2)_6\text{CH}_2\text{CH}_2\text{OH}$ ], and fluorinated ethers. 6:2 FTOH can be degraded in aerobic conditions and its major biodegradation pathways have been elucidated. WWTPs are major sites for 6:2 FTOH-based products to enter the environment. Currently, no information is available on whether 6:2 FTOH can be degraded anaerobically in digester sludge of WWTPs. 6:2 FTUCA [ $\text{F}(\text{CF}_2)_5\text{CF}=\text{CHCOOH}$ ] is a major intermediate of 6:2 FTOH aerobic biodegradation and is also expected to be a major product under anaerobic conditions. Further defluorination of 6:2 FTUCA would lead to various products under both aerobic and anaerobic conditions. This study investigated 6:2 FTOH anaerobic biodegradation and reductive defluorination using 6:2 FTUCA as a model chemical. Titanium (III) citrate/VB<sub>12</sub> mixture is a well established system to study Cobalt I-catalyzed reductive dehalogenation of chemicals via either one-electron transfer or nucleophilic attack of the targeting chemical. The double bond in 6:2 FTUCA molecules allows cobalt I-catalyzed reactions to occur. Digester sludge was collected and diluted with mineral media before dosing with 6:2 FTOH ( $\sim 1.9 \text{ mg L}^{-1}$  final concentration) to carry out anaerobic biodegradation in closed bottles at  $\sim 36^\circ\text{C}$  for up to 6 months.  $\text{O}_2$ -free Titanium (III) citrate/VB<sub>12</sub> solution (pH  $\sim 7.6 - 7.9$ ) was dosed with 6:2 FTUCA ( $\sim 10 \text{ mg L}^{-1}$  final concentration) inside an anaerobic chamber to initiate reductive defluorination reactions. The study details such as setup, conditions, and analytical methods will be discussed. The results show that 6:2 FTOH was transformed to 6:2 FTCA [ $\text{F}(\text{CF}_2)_6\text{CH}_2\text{COOH}$ ], 6:2 FTUCA, and 5:3 acid [ $\text{F}(\text{CF}_2)_5\text{CH}_2\text{CH}_2\text{COOH}$ ] after 6 months whereas little PFHxA was observed, suggesting that anaerobic decarboxylation of 6:2 FTUCA to form perfluorinated carboxylates did not occur. On the other hand, 6:2 FTUCA was rapidly defluorinated and released  $> 4 \text{ mol}$  of fluoride per mol 6:2 FTUCA at day 14. Possible defluorinated products and reductive defluorination pathways for 6:2 FTUCA will be discussed.

**TP074 Mammalian toxicology of short-chain 6:2 fluorotelomer-based products, raw materials and degradation products** R.C. Buck, E. I. duPont de Nemours & Co., Inc. / DuPont Chemicals & Fluoroproducts, DuPont / Chemicals & Fluoroproducts, E. I. duPont de Nemours & Co., Inc. / Chemicals & Fluoroproducts; T. Serex, L. Cox, S. Anand, G. Shawn, M. Russell, M. Himmelstein, DuPont. The global fluorochemical industry is undergoing a significant change moving to short-chain perfluoroalkyl based products which have a more favorable toxicological and environmental profile. A key attribute of the short-chain substances is their rapid elimination in living systems. The change is driven by an industry commitment to curtail manufacture of long-chain perfluoroalkyl acids (PFAAs) such as perfluorooctane sulfonate, perfluorohexane sulfonate and perfluorooctanoic acid and precursor products which may degrade to form them. These and other long-chain substances have been shown to have long elimination half lives in living systems. A comprehensive approach was undertaken to develop a robust mammalian toxicology database for products based on 6:2 fluorotelomer alcohol (6:2 FTOH). The substances studied included polymeric and surfactant products, 6:2 FTOH, 6:2 fluorotelomer methacrylate (6:2 FTMAC), and potential degradation products perfluorohexanoic acid (PFHxA) and 5:3 acid ( $\text{C}_5\text{F}_{11}\text{CH}_2\text{CH}_2\text{COOH}$ ). First tier studies included acute oral and dermal toxicity, single-dose uptake and elimination, genotoxicity and sensitization studies. Higher tier studies on substance classes



followed and included repeated-dose (oral and inhalation) toxicity, and toxicokinetics assessments. This paper will describe the substances evaluated, the tiered study approach and the results of the studies.

**TP075 Aerobic Biodegradation of N-Ethyl Perfluorooctane Sulfonamidoethanol in Soil** S. Mejia, McGill University / Chemical Engineering; J. Liu, University of Maryland Center for Environmental Science / Chesapeake Biological Laboratory, McGill University / Civil Engineering and Applied Mechanics. The environmental fate, global transport and human and ecological health impacts of polyfluoroalkyl substances (PFASs) have been of major scientific interest in recent years. Perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) have been particularly studied due to their persistence and bioaccumulation potential. Their environmental occurrence can arise from direct uses as well as from degradation and transformation of precursors. Overall, there lacks a good understanding of the environmental fate of most precursors, including N-Ethyl Perfluorooctane Sulfonamidoethanol (N-EtFOSE,  $C_8F_{17}SO_2N(C_2H_5)CH_2CH_2OH$ ) and its derivatives. N-EtFOSE had been widely used in protective paper coatings and for production of specialty surfactants prior to its 2002 phase-out in North America, and its production may still continue in Asia. However, its environmental biodegradation potential and its connection with PFOS or PFOA are poorly understood. Our present work focuses on the aerobic soil biodegradation of N-EtFOSE in order to understand the long-term fate and impact of the N-EtFOSE-containing wastes in natural and engineering treatments systems, such as in landfills and wastewater treatment plants. N-EtFOSE and one of its known degradation product, N-ethyl perfluorooctane sulfonamide (N-EtFOSA) are incubated in both open and closed soil microcosms, and extracted for chemical characterization using two types of mass spectrometry. The details on experimental setup, analytical methods and transformation products will be discussed. The proposed degradation pathway will be compared to what is known in aerobic sludge and mammalian metabolism.

**TP076 In-situ partitioning behavior of perfluorinated compounds along a salinity gradient from estuarine and coastal areas of Korea** J. Khim, Korea University / Division of Environmental Science and Ecological Engineering, Korea University / Environmental Science and Ecological Engineering; S. Hong, Korea University / Division of Environmental Science and Ecological Engineering; J. Park, Korea University / Environmental Science and Ecological Engineering; J.E. Naile, U.S. Environmental Protection Agency / Ecosystems Research Division; G.P. Codling, University of Saskatchewan / Department of Veterinary Biomedical Sciences and Toxicology Centre; J.P. Giesy, University of Saskatchewan / Biomedical Veterinary Sciences & Toxicology Centre, Michigan State University / Department of Animal Science, University of Saskatchewan / Toxicology Centre. We investigated the distribution characteristics and partitioning behavior of perfluorinated compounds (PFCs) in surface water ( $n=46$ ) collected from estuarine and coastal areas of Korea. Thirteen individual PFCs in water and suspended solids were quantified by use of HPLC-MS/MS. PFCs were detected in all samples, indicating a widespread distribution in the study area. Great concentrations of PFCs were found at some inland sites which seemed to be affected by direct input of point sources (e.g., wastewater treatment plant) and/or indirect exposure of diffusive sources (such as surface runoff). Spatial distribution of PFCs in estuary along the transect line towards open sea reflected that these chemicals were far transported to outer region due to great amount of discharged water during the rainy season. Looking for the partitioning behavior, the distribution coefficients ( $K_d$ ) for long-chain PFCs (carbon chain  $\geq 8$ ) were significantly correlated with salinity;  $K_d$  values increased exponentially with increasing salinity. It has been shown that such PFCs could be largely scavenged by adsorption onto suspended particles and/or sediments in estuarine environments due to the 'salting-out' effect. In addition,  $K_d$  values of those PFCs generally increased as carbon numbers increased. Salting constants of selected PFCs were notably greater (2-10 fold) than those of other group chemicals, which indicated that adsorption of PFCs is largely associated with salinity. Overall, the results from the present study provided better understanding of fate and transport of PFCs in the field, however, laboratory support would be required to fully identify salting effect in quantitative manner.

**TP077 Riverine discharge loads and emission factors of perfluorinated surfactants (PFSS) estimated by using GIS in Korea** S. Kim, BK-21, School of Earth and Environmental Sciences, Seoul National University /

BK-21, School of Earth and Environmental Sciences, University of Incheon / College of Natural Science, Department of Marine Science. Long-range transport of and exposure to perfluorinated substances (PFSS) strongly rely on their emission mode. In the present study, watershed-based riverine discharge loads and emission factors are estimated for perfluorooctanoate (PFOA), perfluorononanoate (PFNA), perfluorohexylsulfonate (PFHxS), and perfluorooctylsulfonate (PFOS) by using spatially distributed data of concentration and water flow and a geographic information system (GIS). Average per capita emissions (emission factor, mg/capita/day) are 75 for PFOA, 36 for PFNA, 17 for PFHxS, and 43 for PFOS, which are several times lower than the estimates in Japan and the European Continent. A relatively uniform distribution is observed for PFHxS and PFOS emission factors, while the elevated values of PFOA and PFNA dominate in one of eight river basins. This may indicate the predominant contribution of diffusive sources (e.g., nonpoint source) for PFHxS and PFOS in the presence of localized point sources for PFOA and PFNA. The lower-upper bound of total riverine loads discharged annually from the Korean peninsula are in the range of 0.53–1.3 tons for PFOA, 0.09–0.60 tons for PFNA, 0.07–0.29 tons for PFHxS, and 0.19–0.73 tons for PFOS, accounting for < 1% of the global annual emission. Further, the riverine discharge loads are significantly greater than the discharge loads from a wastewater treatment plant, indicating the necessity of further study of a nonpoint source.

**TP078 Emission Factor and National mass loads of perfluorinated compounds (PFCs) discharged from wastewater treatment plants (WWTPs) in Korea** S. Kim, BK-21, School of Earth and Environmental Sciences, Seoul National University / BK-21, School of Earth and Environmental Sciences, University of Incheon / College of Natural Science, Department of Marine Science; K. Zoh, Seoul National University, Seoul National University / Department of Environmental Health, School of Public Health. The discharge of perfluorinated compounds (PFCs) was investigated for 15 wastewater treatment plants (WWTPs), comprising 25% of total domestic wastewater and 23% of total industrial wastewater produced in Korea. PFCs concentrations in influent, effluent, and sludge were greater in industrial wastewater than in the majority of domestic wastewater. Individual PFCs were found to have differing industrial sources, with perfluorocarboxylates used in fabric/textiles, paper-mill, and dyeing industries, and perfluoroalkylsulfonates occurring in oil/chemical and metal-plating/processing industries. Total WWTP-derived national discharge loads were calculated based on the average concentrations in effluents and the total volume of wastewaters produced in Korea. The average WWTP-derived national discharge loads of individual PFCs were 0.04–0.61 ton/year, with 63% of perfluorooctanoate being from domestic wastewater, and 75% of perfluorooctanesulfonate being from industrial wastewater. These estimates accounted for the majority of national emissions, based on measurements in major river mouths, indicating the major contribution of WWTPs to PFC occurrence in Korean aquatic environments. Both the per capita emission factor (mg/capita/day) for domestic discharge, and area-normalized national discharge loads (g/capita/km<sup>2</sup>/day) for all wastewaters were several factors lower in Korea than in Japan or Europe, which is consistent with the lower levels of human exposure to PFCs in Korea.

**TP079 Occurrence of Perfluorinated Compounds in a Lake Receiving Effluents from a Wastewater Treatment Plant** Z. Zhou, State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences; Y. Liang, School of Medicine, Jiangnan University; Y. Shi, Y. Cai, State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences. Perfluorinated compounds (PFCs) have been widely used in industrial and household products due to their extremely thermal and chemical stability, hydro- and lipophobic properties. However, PFCs have been characterized global environmental contaminants, detected in continent, ocean, polar region, wildlife, and human. Recent researches have proved that most prevalent PFCs are persistent, bioaccumulative and toxic in the environment. It has been indicated that industrial and municipal WWTPs are the main point sources for PFCs entering into the aquatic environment. Many studies have investigated the distribution and fates of PFCs during wastewater treatment process. However, few studies have been conducted on the levels and distribution of PFCs in the recipient water that receives effluents discharged from WWTPs. In this study, surface water, sediment, and biological samples were collected from a recipient lake in China to investigate the



effect of WWTP effluent on the concentrations of PFCs in the local aquatic ecosystem. Concentrations of total PFCs in surface water and sediment samples were found in the range of 372–3,495 ng/L and 13.9–659 ng/g dry weight, respectively. High levels of PFHxS, PFOS, and PFOA were found in water samples, with the mean concentrations at 459, 290, and 361 ng/L, respectively. PFOS was the dominant contaminant of PFCs in sediment, with the mean concentration at 125 ng/g dry weight. A decreasing trend of PFOA and PFOS concentrations with increasing distance from the WWTP was found in water and sediment samples, indicating that WWTP effluents was the primary source of PFCs in this region. The distribution coefficient ( $K_d$ ) values were determined for different PFCs in sediment samples, with the range of 4.57–23.8 L Kg<sup>-1</sup> for PFOA and 71.0–1,020 L Kg<sup>-1</sup> for PFOS. The bioaccumulation factor (BAF) generally increased with the chain length in PFCs congeners. The Risk quotients (RQs) of PFOA and PFOS for the surface water suggested that the release of effluents might not pose an immediate risk to the health of the local inhabitants, but the potential risk of the WWTP should not be ignored. In addition, the health risk assessment of PFOS exposure from fish consumption implied that there is potential exposure risk for consumers. However, a more refined risk assessment should be carried out to better understanding the health risks of PFCs to the local inhabitants.

**TP080 Accumulation and Biotransfer of Perfluoroalkyl Acids in Lactating Cows** R. Vestergren, Stockholm University / Applied Environmental Science; F. Orata, U. Berger, I.T. Cousins, Stockholm University.

Beef and dairy products can be important vectors of human exposure to perfluoroalkyl acids (PFAAs) and hence an understanding of the transfer of PFAAs from feed to milk and edible tissues of cows is important for PFAA exposure assessment. In this study, the fate of PFAAs in lactating cows (*Bos taurus*, breed: Swedish Red) exposed to a naturally contaminated diet was investigated by analysis of feed, drinking water, feces, urine and milk samples collected from a barn containing approximately 100 individual adult cows. Distribution of PFAAs to blood, beef and liver was studied in cows which were sent to slaughter by the farmer during the experiment. Perfluorooctanoic acid (PFOA) was the dominant homologue in feed and water samples while perfluorooctane sulfonate (PFOS) was found in the highest concentrations in blood, liver, beef and milk. Biotransfer factors (BTFs), defined as the concentration of PFAA in cow tissues over the intake rate of PFAAs (from feed and drinking water), ranged from 0.03 to 0.36 day kg<sup>-1</sup> in milk and beef for the investigated PFAAs. A positive relationship between BTFs and perfluoroalkyl chain length was observed among the perfluoroalkyl carboxylic acids (PFCAs) with perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA) and perfluorundecanoic acid (PFUnDA) displaying the highest BTF values. Perfluoroalkane sulfonic acids (PFSAs) had higher BTFs compared to (PFCAs) with the same number of perfluorinated carbons which, in line with previous studies, demonstrates that the functional group affects the bioaccumulation potential of PFAAs. Evaluation of the intake rates and measured tissue concentrations employing a one-compartment model suggested that the average whole body elimination half-life of perfluorooctanoic acid (PFOA) was 2 days which is in line with previous experiments in beef cattle using a single oral exposure (1 mg/kg body weight of <sup>14</sup>C-PFOA).

**TP081 Temporal Trends in Dietary Exposure to Perfluoroalkyl Acids for the Swedish Population 1999–2010** R. Vestergren, Stockholm University / Applied Environmental Science; J. Johansson, U. Berger, Stockholm University; A. Glynn, National Food Agency; A. Bignert, Swedish Museum of Natural History; I.T. Cousins, Stockholm University.

Dietary intake has been identified as a major pathway of human exposure to perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS). The conclusion that dietary intake is the major exposure pathway for PFOA and PFOS is, however, associated with large uncertainties due to the scarcity of reliable measurements of these compounds in food and knowledge about how dietary exposure has changed over time. In this study archived food basket samples from 1999, 2005 and 2010 were analyzed using state-of-the-art analytical techniques to estimate total dietary exposure to a range of PFAAs. In addition, farmed rainbow trout, hen's egg yolk and cow's milk samples collected annually over the period 1999–2010 were analyzed to investigate temporal trends of PFAAs in these food items. Total dietary exposure to PFOS (796–1424 pg kg<sup>-1</sup>day<sup>-1</sup>), perfluoroundecanoic acid (88–212 pg kg<sup>-1</sup>day<sup>-1</sup>), perfluorodecanoic acid (52–102 pg kg<sup>-1</sup>day<sup>-1</sup>) and perfluorononanoic acid (62–83 pg kg<sup>-1</sup>day<sup>-1</sup>) was dominated by the consumption of

fish and to a lesser degree of egg and meat. In contrast, dietary exposure to PFOA (322–513 pg kg<sup>-1</sup>day<sup>-1</sup>) was the result of low levels (8–62 pg g<sup>-1</sup>) detected in several high consumption food categories including cereals, dairy products, vegetables and fruit. Statistically significant decreasing temporal trends were observed for PFOS and perfluorohexane sulfonic acid in farmed trout ( $p=0.002$  and  $p=0.032$ , respectively) and hen's eggs ( $p<0.001$  for both compounds). A statistically significant decreasing temporal trend was also observed for concentrations of PFOA in hen's eggs ( $p=0.017$ ). However, no statistically significant temporal trends were observed for perfluoroalkyl carboxylates with 8 to 11 perfluorinated carbons. A dynamic 1-compartment toxicokinetic model was applied to determine if temporal trends in human serum concentrations can be explained by temporal trends in dietary exposure. This demonstrated that present day serum concentrations of PFOS and PFOA in Swedish first time mothers can largely be explained by the intake of contaminated food. However, changes in dietary exposure over the period 1999–2010 cannot explain the decreasing concentrations of PFOS and PFOA in human serum. This indicates that although dietary intake is a major ongoing exposure pathway to PFOS and PFOA other exposure pathways have made a significant contribution in the past.

**TP082 Isomer-Specific Perfluoroalkyl Acid Analysis of Human Serum from Two Cities in North China** Y. Zhang, Nankai University / College of Environmental Science and Engineering; S. Beesoon, University of Alberta / Div. of Analytical and Environmental Toxicology, Department of Laboratory Medicine and Pathology; L. Zhu, Nankai University; J.W. Martin, Trent University / Division of Analytical and Environmental Toxicology.

Isomer-specific analysis of perfluoroalkyl acids (PFAAs) in 129 serum samples from two typical cities (Shijiazhuang and Handan) in North China was conducted. Total PFOS ( $\Sigma$ PFOS, mean 33.32 ng/ml) was the predominant PFAA followed by PFHxS (2.95 ng/ml),  $\Sigma$ PFOA (2.38 ng/ml), and PFNA (0.51 ng/ml). The mean concentrations of total PFAAs ( $\Sigma$ PFAAs) in the participants living in urban Shijiazhuang (59.0 ng/ml) and urban Handan (35.6 ng/ml) were significantly higher ( $p<0.001$  and  $p=0.041$ ) than those living in rural district of Shijiazhuang (24.3 ng/ml). The young females had the lowest  $\Sigma$ PFAAs as compared with the males and old females. On average, the proportion of linear PFOS isomer (n-PFOS) was 48.1% of  $\Sigma$ PFOS, much lower than in technical PFOS from the major historical manufacturer (ca. 70% linear), and also was the lowest proportion compared with other countries. The proportion of n-PFOS decreased significantly with increasing  $\Sigma$ PFOS concentration in the serum samples ( $r=-0.694$ ,  $p<0.001$ ). This may suggest the people with high  $\Sigma$ PFOS could be exposed to disproportionately high levels of PFOS precursors, as previously suggested by exposure models. Linear PFOA (n-PFOA) in the human serum samples contributed 96.1% of  $\Sigma$ PFOA, much higher than in technical PFOA (ca. 75–80% linear), but lower than that in American people. The relatively low proportion of n-PFOA in Chinese people suggests that PFOA manufactured by electrochemical fluorination (ECF) may still be used in China.

**TP083 Temporal Trends in the Enantiomer Fractions of 1m-PFOS in Americans and Swedes** Y. Liu, University of Alberta / Laboratory Medicine and Pathology, University of Alberta; U. Berger, Stockholm University; G. Olsen, 3M; A. Glynn, Swedish National Food Agency; J.W. Martin, University of Alberta / Department of Laboratory Medicine and Pathology, Division of Analytical and Environmental Chemistry.

The wide occurrence of perfluorooctane sulfonate (PFOS) in human serum and the possible associated health risks have attracted scientific interest towards elucidating the various exposure sources and pathways. It has been proposed that both direct PFOS exposure and indirect PFOS-precursor (PreFOS; compounds that can be metabolized to PFOS) exposure can contribute to the total human PFOS body-burdens. However the relative contribution of these exposure pathways remains unclear for most populations. It is also not clear how the relative importance of PFOS and PreFOS exposure might be influenced by gender or age, and how exposure may have changed over time in response to the phase-out by the major historical manufacturer, and the subsequent shift in manufacturing of PFOS and PreFOS to China and other countries. Enantiomer fraction (EF) analysis of chiral PFOS isomers was previously demonstrated in human serum, and this was proposed as a capable tool for evaluating the relative extent of PreFOS and PFOS exposure. Using this established analytical tool, here we examined the alpha-perfluoromethyl branched PFOS (1*m*-PFOS) EF in archived human serum samples from the United States and Sweden. Samples were available from a variety of years in each country, ranging from 1996 to 2010 in Sweden (first time mothers

three weeks after delivery), and from 1974 to 2010 in the United States (adult men and women of various ages). This is the first study elucidating temporal changes in the relative importance of PFOS and PreFOS exposure for humans, and the results were interpreted with regards to determinants of exposure, and known global trends in manufacturing and use of PFOS and PreFOS.

**TP084 Isomer Profiles of Perfluoroalkyl Substances in Paired Samples of Human Serum/Whole Blood and Urine: Renal Clearance Estimation**

**Y. Zhang**, Nankai University / College of Environmental Science and Engineering; **S. BEESON**, University of Alberta / Div. of Analytical and Environmental Toxicology, Department of Laboratory Medicine and Pathology; **L. Zhu**, Nankai University; **J.W. Martin**, University of Alberta, University of Alberta / Laboratory Medicine and Pathology, University of Alberta / Department of Lab Medicine and Pathology, University of Alberta / Department of Laboratory Medicine and Pathology, Division of Analytical and Environmental Chemistry, University of Alberta / Department of Public Health Science. Perfluoroalkyl substances (PFAS) are a group of persistent and bioaccumulative compounds that have been associated with adverse effects in epidemiological studies. Sources of exposure and pathways for elimination in humans are not clear for most PFASs. We aimed to estimate human renal clearance for a range of PFASs and their branched isomers. We conducted a cross-sectional study of PFAS exposure in 86 paired human serum/whole blood and urine samples from general adult residents in China, and we analyzed these samples by isomer-specific liquid chromatography–tandem mass spectrometry. Perfluorohexane sulfonate (PFHxS), perfluorooctane sulfonate (PFOS), perfluorooctanoate (PFOA), perfluorononanoate (PFNA), perfluorodecanoate (PFDA) and perfluoroundecanoate (PFUdA) were detected in > 77% of all samples. We observed that levels in urine were positively correlated with levels in serum/blood for most PFASs. The mean rates of renal clearance (ml serum/day/kg) were lowest for PFHxS < total PFOS < PFUdA < PFDA < PFNA < total PFOA, and one the whole, branched PFOS and PFOA isomers were generally excreted preferentially in human urine, relative to the corresponding linear isomer, however, 1*m*-PFOS was the most persistent PFAS examined.

**TP085 Considerations in Characterizing Residuals in New and Aged Fluorotelomer Polymers**

**J. Naile**, **T. Jenkins**, **J. Washington**, United States Environmental Protection Agency, National Exposure Research Laboratory. Fluorotelomer polymers (FTPs) comprise some of the major products of the fluorotelomer industry. FTPs retain monomer fluorotelomer residuals, e.g., fluorotelomer alcohols (FTOHs), which are known to degrade to perfluorocompounds (PFCs), e.g., perfluorooctanoic acid (PFOA), which are recalcitrant in the environment, bio-accumulative and toxic. Because of the residual monomer content of FTPs, and because FTPs have been shown to degrade to form fluorotelomer monomers, FTPs constitute a source of PFCs to the environment and, consequently, there is a need to characterize the species and concentrations of fluorotelomer and perfluoro compounds in new and aged FTPs. However, FTPs, fluorotelomer compounds and PFCs share the analytically confounding traits of: i) bearing a fluorine-saturated telomer designed to repel non-fluorinated materials; ii) one or more functionalities intended to interact with other molecules or surfaces; and iii) a propensity to remain co-associated with other fluorinated molecules when in the presence of a non-fluorinated matrix. Together, these traits challenge dissolution of the intended analytes during extraction and render the extracted compounds susceptible to loss during handling and analysis. Compounding these challenges, every FTP is unique in terms of structure, consequently the efficacy of any given extraction and analysis methods varies drastically among FTPs, thus effective characterization of FTPs requires unique development efforts for each one. Here we report on some of the challenges we have encountered, variables to consider, and some of the systematics that we use in developing a method to characterize fluorotelomer and perfluoro compounds in a new or aged FTPs.

**TP086 Identification of a Pathway for Perfluorocompounds to Human Diet from Application of Biosolids to Agricultural Fields**

**J. Washington**, United States Environmental Protection Agency, National Exposure Research Laboratory; **H. Yoo**, National Research Council, US EPA / National Exposure Research Laboratory, US EPA; **T. Jenkins**, Senior Service America, US EPA; **L. Libelo**, US EPA / OSCP/OPPT. Perfluoro compounds are ubiquitous contaminants in human blood. The pathways which result in near universal exposure to humans in modern societies are not

clearly understood. Sources to environmental compartments and transport between compartments are only poorly studied, and this lack of data greatly limits any exposure assessment. Biosolids generated by a wastewater treatment plant receiving domestic and industrial (including floorchemical manufacturing-and-use wastewater) effluent were shown to contain levels of perfluorochemicals as high as the parts-per-million range. The biosolids containing elevated levels of per- and poly-fluorinated chemicals were applied to agricultural land in the Decatur, AL area for a number of years. Understanding the potential for human exposure as a result of this land application has required a multiyear effort beginning with the development of analytical methods for a variety of perfluorocarboxylic acids, sulfonates, and precursors in water, sludge, treated biosolids, soil, vegetation and animal tissues. These methods have been used to measure levels of PFCs in environmental media from the initial sludge to agricultural products grown on the receiving soil. High levels of a large number of PFCs and PFC precursors were detected in the tested media. Measured concentrations in each environmental compartment allow the evaluation of the partitioning and transport from the source sludge through to soil to agricultural products. This complete pathway analysis shows that land application of contaminated biosolids can contribute to human exposure. Since all biosolids, that have been tested and reported upon in peer-reviewed literature, contain PFCs and PFC precursors, this exposure pathway represents a potentially significant contribution to humans.

**TP087 The Interaction of Polyfluorinated Compounds with Filter Media: Implications for Phase Partitioning Studies**

**B. Chandramouli**, **C. Hamilton**, **J. Benskin**, Axys Analytical Services, Ltd.. Polyfluoroalkyl compounds (PFCs) including perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) are ubiquitous in the environment. Many PFCs are persistent and their fate in the ecosystem depends on their partitioning between the different phases in the environment. It is therefore important in many cases to be able to accurately measure PFCs after phase separation techniques such as filtration. The unique structures of PFCs with the C-F backbone, their ionisation at neutral pH and surface-active moieties, imply possible adsorption or other interactions with filter media. Significant capture of PFCs by aqueous filters or filtration media has the potential to underestimate PFCs in the aqueous phase and overestimate PFCs in the solid phase. Our study measured the interaction of a suite of PFCs from 6 different PFC classes (perfluoroalkyl carboxylates (PFCAs), perfluoroalkyl sulfonates (PFASs), perfluoroalkyl phosphates (PFPA)s and phosphinates (PFPI)s, polyfluoroalkyl phosphate mono- and diesters (PAPs), fluorotelomer sulfonates, and perfluorooctane sulfonamide) with 4 different types of filter media (nylon, polyethersulphone, Teflon, and glass fibre). Up to three different matrices (drinking water, POTW effluent, and ambient surface water) were tested to study the effects of matrix on filter analyte capture, and up to three different analyte concentration levels were also used to test for concentration effects. The samples were pre-filtered to eliminate particles as a partitioning source, then fortified with a suite of PFCs. After filtration using the test filter media, the filter and filtrate were analysed separately. The analysis was carried out using validated isotope dilution LC-MS/MS methods with SPE cleanup. Results indicate varying levels of adsorption of a number of PFCs, including PFOS and higher carbon PFCs (C10 and up) on all the filter media tested. The polymeric media all showed high and potentially unacceptable levels of adsorption for critical compounds such as PFOS. Sorption of PFPIs and diPAPs ranged from 30-75% regardless of chain length or filter material. Overall, glass fiber filters showed the best performance with negligible filter levels for most PFCs and adsorption < 5% in clean water for PFOS. Preliminary results also indicate that matrix can affect adsorption on filters.

**TP088 Simultaneous characterization of perfluoroalkyl phosphonic/phosphinic acids and polyfluoroalkyl phosphate mono-/diesters in water and whole effluent**

**J.P. Benskin**, **M.B. Woudneh**, AXYS Analytical Services Ltd; **M.G. Ikonomou**, Institute of Ocean Sciences; **J.R. Cosgrove**, AXYS Analytical Services Ltd. Here we report a new analytical method for simultaneous characterization of perfluoroalkyl phosphonic acids (C6, C8, C10 PFPA)s, perfluoroalkyl phosphinic acids (6:6, 6:8, 8:8 PFPI)s, polyfluoroalkyl phosphate monoesters (6:2, 8:2 monoPAPs) and polyfluoroalkyl phosphate diesters (6:2, 8:2 diPAPs) in water and whole effluent by solid phase extraction-high performance liquid chromatography tandem mass spectrometry (SPE-HPLC-MS/MS). These emerging per- and polyfluoroalkyl substances represent a particular challenge to measure in the



environment as they comprise both mono- and di-per/polyfluoroalkyl-substituted species. Furthermore, PFPAs and monoPAPs contain two ionizable protons, making them extremely surface-active. We systematically examined the retention and elution of these substances on weak anion exchange (WAX) cartridges and found that 5mL of 0.3%  $\text{NH}_4\text{OH}$  in either MeOH, ACN or 90:10 MTBE:MeOH was sufficient at eluting di-substituted congeners (PFPis and diPAPs) but not PFPAs or monoPAPs. Only by increasing the concentration of base (4mL of 1%  $\text{NH}_4\text{OH}$  in MeOH) were PFPAs and monoPAPs eluted quantitatively. We also experimented with several mobile phases (acidic, neutral, and basic), and stationary phases (C8, C18, and pentafluorophenyl propyl) and found that a short (5cm) C18 column utilizing a 0.1% ammonium acetate/0.1% acetic acid mobile phase was effective for simultaneous analysis of PFPAs, PFPis, monoPAPs and diPAPs in terms of speed, sensitivity, and peak shape. Method recoveries were excellent for all congeners in water (80-120%) and for mono- and diPAPs in effluent (80-120%) but a wider range was observed for PFPAs and PFPis in effluent (60-140%). Method detection limits were typically  $\sim 5\text{ng/L}$  for PFPAs and monoPAPs and  $< 0.2\text{ng/L}$  for PFPis and diPAPs in 500mL of water or effluent. Initial measurements in whole effluent revealed that 8:2 monoPAP followed by 8:2 diPAP were the dominant congeners, present at concentrations up to  $\sim 25\text{ng/L}$ . To our knowledge this is the first method which is capable of simultaneous characterization of PFPAs, PFPis, monoPAPs, and diPAPs in water and effluent using a single injection.

**TP089 Developments and guidance on measuring PFASs in air using passive and active samplers** L. Ahrens, Environment Canada; T. Harner, Environment Canada / Atmospheric Science & Technology Directorate, Environment Canada / ASTD; M. Shoeb, Environment Canada. The presence of per- and polyfluoroalkyl substances (PFASs) in environmental media all over the world has led to concerns regarding the long term fate, continued long-range transport (LRT), and potential health effects associated with these chemicals. These concerns have led to the recent listing of perfluorooctane sulfonic acid (PFOS) and related compounds (including precursors) under the Stockholm Convention on persistent organic pollutants (POPs). Listing under the Convention requires that monitoring be conducted in air (one of the core media under the Convention) and that these data be reported under the global monitoring plan (GMP). We report on a series of field-based studies that have evaluated passive and active air sampling of these chemicals in order to improve guidance for the global monitoring plan. Our findings show that polyurethane foam disk passive air samplers (PUF-PAS) can be used for measuring PFOS and precursors in air but have limited sampling capacity for some other PFASs. Sorbent-impregnated polyurethane foam (SIP) disk PAS have been demonstrated to be a feasible alternative for the more volatile PFASs such as fluorotelomer alcohols (FTOHs). However, when shorter time resolution is required and for the determination of gas-particle partitioning, high-volume active air samples (HV-AAS) are typically used. The current study highlights some of the sampling artifacts associated with conventional HV-AAS when measuring perfluoroalkyl carboxylic acids (PFCAs) and perfluoroalkanesulfonic acids (PFASs). Gas-phase compounds were shown to accumulate on the glass fiber filter (GFF) by adsorbing to particulate matter already collected on the GFF, resulting in an overestimation of the particle-phase concentration ("blow-on" artifact). Results are presented from active air sampling using an alternative, denuder sampler that avoids sorption to the GFF by capturing the gas-phase first. The denuder results are used to investigate the relationship between  $pK_a$  and particle-gas partition coefficient ( $K_p$ ) for the PFASs. The findings highlight the importance of aerosol pH and the need to develop particle-gas partitioning models for ionizable chemicals, and for accurate determination of their  $pK_a$  values. Overall, this study reinforces the air sampling and analytical challenges that are unique to the PFASs.

**TP090 Perfluoroethylcyclohexanesulfonate (PFECHS) and perfluorinated sulfonates and carboxylates in snapping turtles from southern Ontario, Canada** R.J. Letcher, Carleton University / Department of Chemistry, Environment Canada / Science and Technology Branch, Ecotoxicology and Wildlife Health Division, Ecotoxicology and Wildlife Health Division, Science and Technology Branch / Environment Canada; S. Chu, Ecotoxicology and Wildlife Health Division, Science and Technology Branch / Environment Canada; S.R. de Solla, Environment Canada / Science and Technology Branch; A.O. De Silva, Environment Canada / Water Science & Technology Directorate, Environment Canada / Water Science and Technology. Perfluoroethylcyclohexanesulfonate (PFECHS) is a cyclic

perfluoroalkyl acid (PFAA) mainly used as an erosion inhibitor in aircraft hydraulic fluids. Usage of PFECHS in Canada and the United States is permitted based on the lack of alternatives for the safe performance of aircraft and the purported minimal environmental release. However, PFECHS was recently reported in whole Lake trout samples and surface water samples from Lakes Superior, Huron, Erie and Ontario. We also recently reported PFECHS in surface water, amphipods and shrimp from the Welland River and Lake Niapenco in southern Ontario, Canada. The headwater of the Welland River is adjacent to and on the property of the John C. Munro International Airport, Hamilton, Ontario, Canada. PFECHS appeared to be associated with the airport as a source as it was present in amphipods immediately downstream of the airport on the Welland River, but declined to almost zero about 50 km downstream. Plasma was also collected in summer 2007 to 2010 from snapping turtles (*Chelydra serpentina*) from Lake Niapenco East and West near the airport, and also from turtles from other southern Ontario sites. At the time, various perfluorinated carboxylates and other sulfonates (including perfluorooctane sulfonate (PFOS)) were determined, but not PFECHS. In the present study, we report on PFECHS in plasma of snapping turtles from these southern Ontario sites, and compare the levels of PFECHS precursors and to other perfluorinated carboxylates ( $\text{C}_6$  to  $\text{C}_{14}$  chain lengths) and sulfonates ( $\text{C}_4$ ,  $\text{C}_6$ ,  $\text{C}_8$  and  $\text{C}_{10}$  chain lengths). PFOS was by far the most concentrated PFAA, with mean concentrations as follows: Lake Niapenco (East;  $n=9$ )  $2377 \pm 1460$ , Lake Niapenco (West;  $n=9$ )  $2065 \pm 650$ , Upper Welland River ( $n=11$ )  $2284$ , Credit River ( $n=10$ )  $171 \pm 120$ , Cootes Paradise ( $n=7$ )  $53 \pm 17$ , Humber River ( $n=7$ )  $121 \pm 90$  and Island Lake ( $n=4$ )  $15 \pm 4$  ng/g ww. For PFECHS the concentration ranges were: Lake Niapenco  $0.48 - 1.48$ , Upper Welland River  $1.12 - 3.12$ , Credit River n.d. -  $1.84$ , Cootes Paradise n.d. -  $0.23$  and Humber River  $0.20 - 1.26$  ng/g ww. The spatial trends of PFECHS appear to be similar to PFOS in snapping turtle plasma as well as PFECHS in amphipods and water samples from the same sample sites. PFECHS contamination in the L. Niapenco area may have been from a point source and the result of hydraulic fluid release and/or had originated as a component of PFOS-based, aqueous film forming foams, which is used by commercial airports as fire suppressants.

**TP091 Bioaccumulation of Perfluorooctane Sulfonate (PFOS) After Laboratory Exposure to Sediments Collected in Ontario** T. Watson-Leung, Ontario Ministry of the Environment / Aquatic Toxicology Unit, Ontario Ministry of the Environment / Aquatic Toxicity Scientist, Ontario Ministry of the Environment / Sediment Toxicologist; S.P. Bhavsar, Ontario Ministry of the Environment. After the discovery of high concentrations of PFOS in snapping turtle plasma in a rural reservoir located downstream of a firefighting training facility, investigations into sport fish concentrations led to the first ever restrictive sport fish consumption advisory for this substance in Ontario. PFOS is perhaps the most well-known of the perfluorochemicals (PFCs), which are man-made chemicals used in commercial and consumer products, and in a variety of industrial applications. PFOS has several uses, including: a water, stain and oil repellent for textiles, carpet, and food packaging; a surfactant in the electroplating industry; and, an additive in firefighting foams. PFOS is both persistent in the environment, and well absorbed and poorly eliminated in biota. It does not behave like lipophilic biomagnifying compounds but instead undergoes enterohepatic recirculation, distributing throughout blood, muscle and liver cells. It was found that there is no relationship between fish size and tissue PFOS concentrations in sport fish collected from the area. As such it was of further interest to assess bioaccumulation of these compounds under controlled laboratory conditions. A 28-day laboratory bioaccumulation test was conducted on sediments collected from four sites along a downstream gradient from the firefighting training facility, as well as laboratory control sediment. PFOS accumulation was assessed in two invertebrate species (the burrowing mayfly, *Hexagenia limbata*, and freshwater oligochaete, *Lumbriculus variegatus*) and one warm-water fish species (the Fathead Minnow *Pimephales promelas*). Organisms were purged for 24 hours after the 28-day exposure and tissue concentrations of PFCs were determined. The movement of PFOS from sediment into laboratory dilution water was assessed through time series water sampling.

**TP092 Novel and Legacy Fluorsurfactant Contamination in Ground-water from Aqueous Film Forming Foam (AFFF) use at Military Bases** W.J. Backe, Oregon State University / Department of Chemistry; J.A. Field, Oregon State University / Environmental and Molecular Toxicology. Legacy per- and poly- fluorosurfactants, such as PFOS and PFOA, are well studied



environmental contaminants due to their ubiquity and persistence. Aqueous film forming foams (AFFF), which contain a complex mix of fluorosurfactants, are used to extinguish hydrocarbon fires and were employed at military bases for fire-training exercises. Elevated concentrations of PFOS, PFOA, 6-2 FtS and their homologs, are measured in groundwater at military sites where fire-training exercises occurred. A recent study has revealed the existence of previously unstudied classes of fluorosurfactants in AFFF formulations that are stored at military bases. It is likely that these newly-identified fluorosurfactants also contaminate groundwater at sites where AFFFs were used and that previous studies have underestimated the breadth of fluorosurfactant contamination. The purpose of this study was to develop a comprehensive method to analyze new and legacy fluorosurfactants in groundwater by HPLC-MS/MS and apply that method to groundwater samples from military sites. The method detection limits are in the sub to single ng/L range and newly-identified fluorosurfactants are shown to co-occur in groundwater.

**TP093 Polyfluorinated amides: a novel indirect PFCA source by electrochemical fluorination of sulfonyl fluorides** D.A. Jackson, University of Toronto / Department of Chemistry, University of Toronto; S.A. Mabury, University of Toronto / Department of Chemistry. One aspect of fluorochemical research that has not been fully understood is the global dissemination of electrochemical perfluorooctanoate (PFOA). Branched isomers of PFOA have been detected in most environmental media from remote Arctic lakes to human blood; their presence may not be adequately explained by direct emissions of ECF PFOA. In the present study we introduce a completely unexplored class of polyfluorinated compounds that can serve as indirect and branched PFOA precursors. Polyfluorinated amides (PFAMs) are a class of compounds produced as byproducts of polyfluorinated sulfonamide synthesis by ECF. In the present study, four PFAM derivatives of PFOA were screened for in a wide range of compounds, experimental materials, and commercial products synthesized by ECF. Initial screening was performed using SPME-GC-MS and quantification using in-house synthesized standards was accomplished with GC-MS using positive chemical ionization. Two monosubstituted PFAMs, *N*-methylperfluorooctanamide (MeFOA) and *N*-ethylperfluorooctanamide (EtFOA), were detected in the majority of materials that were analyzed. The concentrations of PFAMs in the sulfonamide compounds under study ranged from 12 ppm – 6736 ppm (m/m) and every sample included an isomer profile. Both observations suggested their historical importance as an indirect ECF PFOA source. A hydrolysis study performed at pH 8.5 showed no degradation of EtFOA to PFOA after 8 days due to the stability of the amide bond. Therefore, the environmental fate of PFAMs is suggested to be volatilization to the atmosphere followed by oxidation by hydroxyl radical with a predicted lifetime of 3 – 20 days. Subsequent PFAM exposure to biota will likely lead to enzymatic hydrolysis of the amide linkage to give a PFCA.

**TP094 Liquid-Liquid Microextraction LC-MS/MS Analysis of Polyfluorinated Chemicals in Landfill Leachate** B. Allred, Oregon State University / Department of Chemistry; J. Field, Oregon State University / Department of Environ. & Molecular Tox.; M. Barlaz, J. Lang, North Carolina State University / Department of Civil, Construction, and Environmental Engineering. Municipal landfills are part of the next generation of hazardous waste sites. The leachate coming from landfills is a highly complex matrix containing a myriad of compounds of environmental concern, including perfluorinated chemicals (PFCs). To understand the conditions influencing the extent of PFC leaching, a faster and more economical method for leachate sample clean up and PFC analysis has been developed with equal sensitivity to past SPE based methods. In collaboration with Dr. Barlaz at NCSU this method will be used to survey landfills across the United States, as well as study abiotic leaching versus biodegradation. Liquid-liquid microextractions were optimized for pH, surface disruption, volume, ionic strength and matrix removal. Optimal conditions involved titrating 3 ml sample aliquots to a pH of 1.5, using an ethyl acetate to disrupt surface foaming, centrifugation and collecting 3 replicate ethyl acetate extracts. The collected extracts were diluted to 1.5 ml with methanol. The LC-MS/MS method was optimized for large volume (900 µl) injection, the normal phase loading column and gradient elution. The extract was loaded onto dual diol size exclusion guard cartridges (4.6 x 12.5 mm, 5 µm each) and separated with a C18 analytical column (4.6 x 75 mm, 3.5 µm) Detection was achieved with electrospray ionization and triple quadrupole mass spectrometry. The LOD for a suite of >50 analytes (both anionic and neutral) were

at low ng/L levels. Linearity was over 3 orders of magnitude. The NCSU bioreactors used to simulate landfill degradation were tested for background PFC contributions under clean, acidic and anaerobic conditions.

**TP095 Factors Affecting the Distribution of Perfluorinated Compounds in Sediments from Lake Shihwa, Korea** J.E. Naile, U.S. Environmental Protection Agency / Ecosystems Research Division; H. Yoo, National Research Council, US EPA / National Exposure Research Laboratory, US EPA; T. Jenkins, Senior Service America; K. Lee, Institute of Environmental Protection and Safety; J.P. Giesy, University of Saskatchewan / Biomedical Veterinary Sciences & Toxicology Centre, Michigan State University / Department of Animal Science, University of Saskatchewan / Toxicology Centre; J. Washington, United States Environmental Protection Agency, National Exposure Research Laboratory. Perfluorinated compounds (PFCs) are ubiquitously distributed in various environmental media including water, soil, sediment, and biota. PFCs have also been shown to biomagnify in both aquatic and terrestrial environments. Lake Shihwa is an artificial saltwater lake, located on the west coast of Korea, which has been receiving industrial wastewater discharges from the Shihwa and Banweol Industrial complexes. Previous studies have reported elevated levels of PFCs in both water and biota samples. Sediment samples were collected from inside and outside the Lake Shihwa industrial complex, where some of the highest water concentrations ever measured have been reported. Nine perfluorocarboxylate acids (PFCAs), five perfluorosulfonate acids (PFSAs) and six precursors (amides, acetates, and fluoro telomers) of perfluorooctane sulfonate (PFOS) were surveyed using UPLC-MS-MS. In addition to determining sediment contamination profiles for PFCs and their precursor, other sediment physical characteristics were measured to understand the key factors affecting distribution dynamics of these halogenated chemicals in this semi-closed artificial sea-lake environment. Interestingly, sampling locations where high waterborne or biota contamination had been previously reported coincided with highly contaminated sediment sites, supporting the existence of local point sources. Concentrations of PFCs ranged from below the method detection limit (MDL=150 pg/g) to as high 246,805 pg/g dry weight. Two fluorotelomer alcohols (6:2FTS, 8:2 FTS) ranged from below the MDL (=100 pg/g each) to as great as 277 and 226 pg/g, respectively. In addition, two sulfonamides (sFOA, FOSAA), and two acetates (methylated, ethylated) were found at levels as high 32,172 pg/g (EtFOSAA) and 2,006 pg/g (MeFOSAA). The log-transformed distribution coefficient  $K_d$ , was found to be significantly associated with fluorinated carbon number (0.18 increment per pair of C-F<sub>2</sub> unit addition). Overall this study provides useful field based distribution data for a wide variety of PFCs, which will be useful for future modeling efforts.

**TP096 Polyfluoroalkyl and perfluoroalkyl substances in the atmosphere and marine waters of the Canadian Archipelago** A.B. Gawor, University of Toronto / Departments of Chemistry and Physical and Environmental Sciences; L. Ahrens, Swedish University of Agricultural Sciences / Department of Aquatic Sciences and Assessment; M. Shoeib, Environment Canada / Atmospheric Science and Technology Directorate; L.M. Jantunen, Environment Canada. Per and polyfluoroalkyl substances (PFAS) have gained global attention for their bioaccumulative, persistent, long range transport potential and toxicity, particularly PFOS and PFOA. Recently, PFOS and its salts was added to Annex B of the Stockholm Convention on Persistent Organic Pollutants (POPs). Accordingly, industries have changed from producing the long chain PFAS towards shorter chain compounds. The purpose of this study was to determine the levels of PFAS, particularly for PFCAs and PFSAs in Canadian Arctic air and ocean water. Concurrent air and surface water samples were collected in addition to water depth profiles on board the CCGS *Amundsen* in the Canadian Archipelago during August 2010 and October 2011. In the air samples, five PFAS groups (fluorotelomer alcohols FTOHs, fluorotelomer acrylates FTAs, fluorinated sulfonamides FOSAs, fluorinated sulfonamidoethanols FOSEs, perfluorocarboxylates PFCAs, and perfluoroalkyl sulfonates PFSAs) were quantified on both the particulate (glass fibre filters) and gaseous phases (PUX/XAD-2 sandwiches). In the water samples, two of the groups were quantified, PFCAs and PFSAs. Dominant PFAS in the gaseous phase was FTOH, whereas on particulates, FOSEs and PFBA were the dominant PFAS detected. In water, concentrations did not change with depth for the upper 50-100 meters. Concentrations in water were the highest for PFCAs, particularly for PFBA, PFPeA and PFNA.

**TP097 Probing Heat-activated Persulfate Defluorination of Perfluorooctanoate Under Conditions Relevant to Military Fire-Fighting Areas** S. Park, Purdue University / Agronomy, Purdue University; L.S. Lee, Purdue University / Dept. of Agronomy Crop, Soil & Environmental Sciences, Purdue University / Dept. of Soil, Crop & Environmental Sciences; S.A. Waisner, U.S. Army Engineer Research and Development Center; V.F. Medina, US Army Corps of Engineers-Engineer Research and Development Center / Environmental Laboratory; J. Mattei-Sosa, U.S. Army Engineer Research and Development Center. Polyfluoroalkyl substances (PFASs) have been widely used in industrial and commercial application for about 50 years as surfactants, emulsifiers, stain resistant coatings, fire retardants, and polymer additives. The PFASs of greatest current concern in the environment are perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA), which have been shown to be environmentally persistent, bioaccumulative and potentially toxic. PFOS and PFOA may enter the environment through direct discharges, from residuals in commercial products, or as terminal end products of the microbial degradation of other PFASs. PFASs are also commonly found in Aqueous Fire Fighting Foams (AFFFs), which have been demonstrated to be highly effective at fighting fires associated with petrochemicals. Most military facilities that routinely service aircraft maintain fire-fighting training areas to allow emergency personnel to train with AFFFs. Training has resulted in the release of these persistent compounds in soil, leachate, and groundwater. Due to their exceptional stability, many conventional techniques including biological degradation, oxidation and reduction are not effective at destroying PFOS and PFOA in ambient environments. The current study explores the optimal in-situ operating conditions for using heat-activated persulfate for defluorinating environmentally relevant concentrations of PFAS (10 to 1000 ug/L) that are present in groundwater associated with military sites. We quantify the effect of temperature 20 °C – 60 °C, persulfate concentration (1,000 to 20,000 mg/L), reaction time (up to 225 hours for lower temperatures), and the presence of other constituents common at these sites (e.g., chlorinated solvents and fuel constituents) on the efficacy of persulfate to defluorinate PFOS and PFOA. The apparent removal of PFOA increased with increasing temperature with 75% removal at 40 °C in 138 hours and complete loss of PFOA at 50 °C in 72 hours and at 60°C in 48 hours. Increasing initial persulfate concentrations at 50 °C resulted in increased rate of PFOA degradation. Low level metabolites of perfluorobutanoic, perfluoropentanoic, perfluorohexanoic, and perfluoroheptanoic acids were generated and then subsequently degraded. Analysis of fluoride using both ion chromatography and an F-specific electrode was challenging due to the large amounts of sulfate present. PFOS did not appear to be altered by persulfate oxidation.

**TP098 Concentrations of Perfluorinated Compounds in Wastewater Effluents and Surface waters in New England, Massachusetts** K. Dasu, Purdue University / Department of Agronomy, Crop, Soil and Environmental Sciences, NRC Post Doc at US EPA / Office of Research and Development, National Risk Management Research Laboratory, U.S. Environmental Protection Agency / National Risk Management Research Laboratory; S.F. Nakayama, National Institute for Environmental Studies / Centre for Environmental Health Sciences, National Institute for Environmental Studies / Center for Environmental Health Sciences; M.A. Mills, U.S. EPA, U.S. EPA / National Risk Management Research Laboratory, ORD, US EPA / Remediation and Pollution Control Division, NRMRL, U.S. EPA / Office of Research & Development; R. Marfil-Vega, ORISE Postdoc at US EPA / ORISE Research Participant to the National Risk Management, University of Cincinnati, ORISE Postdoctoral Fellow, ORD, US EPA, ORISE Postdoc at US EPA; K. Tadele, Student Service Contract at USEPA. Perfluorinated compounds (PFCs) are ubiquitously detected in human blood, wild life and different environmental matrices such as soils, surface waters, wastewater treatment plant (WWTP) effluents and biosolids. Due to the unique hydrophobic, oleophobic and stain resistant properties, PFCs have wide range of commercial applications as non-stick coatings on carpets, fabrics, cookware, popcorn bags also used in fire fighting foams. PFCs are persistent, bioaccumulative and are shown to be toxic in laboratory animals. Wastewater treatment plants are one of the point sources of PFCs to the water cycle. During the last decade, PFCs are monitored extensively in different environmental matrices including WWTP effluents and biosolids. With the recent reduction in direct emissions and the efforts for the complete elimination of perfluorooctanoic acid (PFOA) and higher homologues in the product content by major manufacturers under 2010/2015 PFOA Stewardship program, there is an increasing interest

to monitor for changes if any, in the concentrations and composition of different PFCs in environmental samples. In the current study, monitoring of PFCs is done as part of a multi-laboratory and interdisciplinary effort to evaluate the endocrine disrupting potency of wastewater effluents. The study monitors for eleven perfluoro carboxylic acids (PFCAs) and four perfluoroalkyl sulfonates (PFSA) in 24 hr composite samples from four wastewater treatment plant effluents in two sampling events. The grab samples from four surface waters downstream and one upstream of those WWTPs were also monitored at one sampling event. Water samples were extracted using solid phase extraction cartridges and analyzed on the ultra performance liquid chromatography electrospray ionization tandem mass spectrometry. The data on the current occurrence, concentrations and distribution of PFCAs and PFSA of different chain lengths will be presented.

**TP099 Development of Watershed-Specific Risk-Based DDT Sediment Benchmarks for the Protection of Fish** P.C. Fuchsman, ENVIRON International Corporation, ENVIRON International Corp.; E. Perruchon, M. Ferguson, M. Nielsen, ENVIRON International Corp.. As part of the identification of preliminary remediation goals (PRGs) for DDT and its metabolites in river sediments, we developed risk-based sediment concentrations specifically for the protection of fish. The derivation approach included: 1) identifying a tissue-based toxicity reference value based on published studies linking whole-body fish tissue concentrations and toxic effects; 2) identifying species-specific exposure factors, including whole fish lipid content and area use factors; and 3) integrating the species-specific exposure factors with chemical-specific bioaccumulation factors to predict a total DDx concentration in sediment that would result in fish tissue concentrations equal to the toxicity reference value. Using a previously published tissue-based toxicity reference value (Beckvar et al. 2005, ETC 24:2094), the PRG to protect fish was more conservative than PRGs derived to protect benthic invertebrates or human health. However, review of the original studies identified by Beckvar et al. (2005) linking DDx bioaccumulation to effects on fish revealed that several were inappropriate for identifying sediment PRGs due to misclassification of life stage, misidentification of DDx component, irreproducible test results, questionable relevance of endpoints, and small magnitude of effect. We reviewed the remaining available studies to identify an alternative, conservative fish tissue toxicity reference value for DDx. Bioaccumulation of DDx in fish was estimated from a local fish tissue monitoring survey. A risk-based DDx sediment goal to protect fish was extrapolated from the tissue effect residue using the watershed-specific TOC- and lipid-normalized biota-sediment accumulation factor. Based on the reanalysis of DDx toxicity in fish, the site-specific PRGs to protect fish were essentially equal to those for protection of benthic invertebrates and human health.

**TP100 Extension of the Target Lipid Model to soils and sediments using Equilibrium Partitioning** A. Redman, Hydroqual, Exxon Mobil Biomedical Sciences; M. Paumen, ExxonMobil Petroleum and Chemicals; T. Parkerton, ExxonMobil Biomedical Sciences, Inc; J. McGrath, HDR; D. Di Toro, University of Delaware; S. Fiebig, G. Winklemann, NOACK-Laboratorien; M. Webley, AstraZeneca; C. Eadsforth, SHELL. The Target Lipid Model (TLM) provides a framework for deriving predicted no effect concentrations (PNEC) for nonpolar organic chemicals. This approach has been used to perform environmental risk assessment of individual hydrocarbons as well as complex petroleum substances. The TLM is based primarily on data for aquatic test organisms and this work evaluates the potential for extending the TLM to soil and sediment using Equilibrium Partitioning (EqP) theory. Literature data for hydrocarbons and other nonpolar organics were compiled for acute and chronic exposures in soils and sediments for invertebrates and plants. New toxicity data were developed to evaluate soil and sediment dwelling organisms and to test potential toxicity cutoffs for high logKow compounds. The default TLM and TLM-derived PNEC were applied to these data using EqP to develop critical body burdens (CBB) including associated uncertainty in the model application. Comparison of the CBBs for soil and sediment species to CBBs from the larger TLM database for aquatic organisms showed no difference in the relative sensitivity between the two groups of species within the uncertainty of the model and experimental data. Furthermore, the acute to chronic ratios (ACRs) for soil and sediment tests were within the range of ACRs for aquatic organisms exposed to nonpolar organic chemicals. The TLM-derived PNEC applied to these data, also, demonstrated sufficient level of protection (e.g., ~95% of data above PNEC) even for chemicals up to logKow 6. For chemicals with



logKow >6 we observed an increasing incidence of no toxicity consistent with the dataset for aquatic organisms. For high logKow chemicals, the duration of the pre-equilibration step was important. For example, toxicity was observed for these chemicals following short pre-equilibration times (< 2 days), whereas no toxicity was observed for spiked soils that had been aged up to 7 weeks prior to exposure. The use of EqP with TLM-derived endpoints is suitable for risk assessment purposes.

**TP102 Prediction of large-scale temporal-spatial distribution of pesticides for paddy field and comparison with field survey results performed in Japan** Y. Imaizumi, F. Shiraishi, S. Serizawa, National Institute for Environmental Studies; J. Goukon, Miyagi Pref. Inst. Pub. Health & Env.; Y. Imazu, Shizuoka Inst. Env. & Hygiene; R. Nagai, Kagoshima Pref. Inst. Env. Res. & Pub. Health; K. Kawata, Niigata University of Pharmacy and Applied Life Sciences; H. Yamamoto, The University of Tokushima; D. Nakajima, T. Sakurai, N. Suzuki, H. Shiraishi, National Institute for Environmental Studies.

Ecological risks are usually assessed based on the ratio between the predicted environmental concentration (PEC), which is usually calculated based on a fate model or survey results, and the predicted no effect concentration (PNEC). Pesticides are characteristically used at limited time points and places, hence for environmental risk assessment it is important to predict temporal-spatial variations of the exposure concentrations. The multimedia environmental fate model G-CIEMS (Grid-Catchment Integrated Multimedia Modeling System) based on Japanese GIS data was developed in the past. In addition, emission estimation method PeCHREM (Pesticide Chemicals High Resolution Estimation Method) for herbicides used typically in paddy fields were developed, that were applied to calculate temporal-spatial variations of herbicides concentrations in river waters for the total area of Japan. The model simulations were validated using field survey data from seven rivers. In this study, we focused on expanding target pesticides not only herbicides but also fungicides and insecticides. The model simulations were validated using field survey data from seven rivers. The emission estimation method was divided into following three phases. In the first phase, applied amounts of each pesticide formulation, which is the commercially available pesticide product, were predicted for each day for each prefecture. In the second phase, we predicted variations of concentrations for each pesticide in paddy fields and daily emission ratios into rivers and atmosphere. In the third phase, we allocated emission amounts of pesticides to GIS segments based on land use data. Finally we calculated environmental concentration for all pesticides for the total area of Japan by the G-CIEMS model. We performed field survey from March 2011 to September 2011. In this field survey three target pesticides (azoxystrobin, dinotefuran, flutolanil) were quantified by LC/MS/MS analyses. The maximum concentrations and the maximum days, when concentrations come up to the maximum value, were compared between predictions and observations for the 20 pairs from total 21 pairs (= 7 river sites x 3 pesticides). For the maximum concentrations, the 11 pairs had a prediction error of less than one order of magnitude and the other 7 pairs had that of approximately one order of magnitude. On the other hand, the prediction accuracy for the maximum days was low.

**TP103 San Jacinto River Waste Pits, Texas, Part-1: Analysis of Polychlorinated Dibenzo-p-dioxins, Furans, and Biphenyls in Surficial River Sediment** L. Aguilar Lazaro, Baylor University / Environmental Science; B. Subedi, Baylor University / Department of Chemistry and Biochemistry, Baylor University / Department of Biology, Baylor University / Department of Chemistry and Biochemistry, Baylor University / Department of Environmental Science, Baylor University / Department of C; S. Williams, Baylor University, Baylor University; J. Berninger, Baylor University / Department of Environmental Science; B.W. Brooks, Baylor University / Environmental Science and Biomedical Studies, Baylor University / Department of Environmental Science; S. Usenko, Baylor University / Department of Environmental Science, Baylor University / Department of Chemistry and Biochemistry.

The San Jacinto River Waste Pits are located on the Houston Ship Channel, Texas. The waste pits were utilized in the 1960's-70's to dispose of pulp and paper waste. Previous studies have identified dioxins, furans, and dioxin-like polychlorinated biphenyls (dl-PCBs) in the Houston Ship Channel. The river waste pit site was placed on the US EPA National Priority List of Superfund sites in 2008. Remedial investigations and feasibility studies began in 2010. Prior to remediation efforts dioxins, furans, and dioxin-like PCBs were measured in surface sediment samples collected at 15 sites in the San Jacinto River Waste Pits site. Aliquots of surficial

sediment from each sampling site were used for characterization of physical parameters including percent moisture, total organic carbon, and percent black carbon. Dioxins, furans, and dl-PCBs were extracted from sediment samples using an enhanced pressurized liquid extraction technique. Extracts were analyzed using a high-resolution gas chromatography-electron capture negative chemical ionization mass spectrometry. The distribution and profile of the individual contaminants at the site were examined through multivariate statistical analysis. The concentration of contaminants exhibit in general the following pattern: total dl-PCBs > total dioxins > total furans and total contaminant concentrations extended two orders of magnitude (~1,800 to ~488,000 pg g<sup>-1</sup> dw). Spatial analysis of contaminant concentrations was further investigated utilizing percent total organic carbon or black carbon normalization.

**TP104 San Jacinto River Waste Pits, Texas, Part-2: Polychlorinated-dioxins, Furans, and Biphenyls in Fish, Crabs, and Clams** B. Subedi, Baylor University / Department of Chemistry and Biochemistry, Baylor University / Department of Biology, Baylor University / Department of Chemistry and Biochemistry, Baylor University / Department of Environmental Science, Baylor University / Department of C; S. Williams, Baylor University / Department of Environmental Science; J. Berninger, Baylor University; L. Aguilar, Baylor University / The Institute of Ecological, Earth, and Environmental Sciences; B. Brooks, S. Usenko, Baylor University / Department of Environment Science.

Fish, crabs, and clams were collected from the San Jacinto River Waste Pits, a Superfund site in Houston, TX, and analyzed for polychlorodibenzo-p-dioxins, polychlorodibenzofurans (PCDD/Fs) and dioxin like polychlorobiphenyls (dl-PCBs). Sample preparations proceeded through tissue homogenization, enhanced pressurized liquid extraction and cleanup, and concentration. Isotopically-labeled surrogates were spiked prior to extraction and were used to correct for target analytes loss during sample preparation. Samples were analyzed employing gas chromatography negative chemical ionization mass spectrometry as well as high-resolution mass spectrometry. The method detection limits ranged from 2.0 to 10 pg/g ww in fish tissues. Ten out of twelve high priority dl-PCBs were measured concentrations ranging from 2.0 to 948 pg/g ww. Average dl-PCBs concentrations were at least an order of magnitude higher than that of PCDD/Fs. Average PCDD/Fs fish tissue concentrations were at least an order of magnitude higher than that of crabs and clams. The chemical profile of mono-ortho-dl-PCB congeners (PCB-105, 114, 118, 123, 156, 157, 167 and 189) in fish, crabs and clams were similar to that in sediments; however, that of non-ortho-dl-PCB congeners (PCB-77, 81, 126 and 169) as well as PCDD/Fs in fish, crabs and clams were different than in sediments.

**TP105 San Jacinto River Waste Pits, Texas, Part-3: Delineation of the Aquatic Food Web of the Site** S. Williams, Baylor University, Baylor University; J. Berninger, Baylor University / Department of Environmental Science; B. Subedi, Baylor University / Department of Chemistry and Biochemistry, Baylor University / Department of Biology, Baylor University / Department of Chemistry and Biochemistry, Baylor University / Department of Environmental Science, Baylor University / Department of C; L. Aguilar Lazaro, Baylor University / Environmental Science; M. Dekar, Baylor University; S. Usenko, Baylor University / Department of Environmental Science, Baylor University / Department of Chemistry and Biochemistry; B.W. Brooks, Baylor University / Environmental Science and Biomedical Studies, Baylor University / Department of Environmental Science.

From the late 1960s to the early 1980s, process sludge from paper manufacturing was deposited on a site adjacent to the southern San Jacinto River. Over time, dredging in the area promoted subsidence of the site, which in turn caused contamination and spreading of polychlorinated dibenzo-p-dioxins and -furans into the surrounding environmental media. Ongoing monitoring of edible fish and crabs in the Houston Ship Channel area documented high concentrations of PCDD/Fs and PCBs that prompted consumption advisories for the region and led to the site's addition to the National Priorities List. A primary goal of our ongoing project is to use isotopic discrimination to characterize the aquatic food web likely to be impacted by the movement of PCDD/Fs and PCBs from the San Jacinto River Waste Pits site. Thirteen aquatic species were collected at the site in three sampling rounds. Among the species collected were redbfish (*Sciaenops ocellatus*), black drum (*Pogonias cromis*), speckled trout (*Cynoscion nebulosus*), and blue crab (*Callinectes sapidus*), which are most often caught and consumed by persons in the area. Tissue samples from each of these thirteen species were dried and subjected to isotope analysis for carbon and nitrogen; the resulting data



was used to assign trophic positions for each species. This process was also informed by information on life history, feeding behaviors, and seasonal dynamics. The outcome of our analysis is also compared to previous studies in nearby ecosystems that are expected to be similar. An understanding of the complexity of the food web, in conjunction with chemical analysis for PCDD/Fs and PCBs, will ultimately provide an avenue for characterization of food-web specific patterns of bioaccumulation of these persistent organohalogen pollutants that may be applicable to other sites with similar profiles of contamination.

**TP106 San Jacinto River Waste Pits, Texas, Part-4: Food-web Specific Patterns of Bioaccumulation for Polychlorinated Dioxins, Furans, and Biphenyls** S. Williams, Baylor University, Baylor University; J. Berninger, Baylor University / Department of Environmental Science; B. Subedi, Baylor University / Department of Chemistry and Biochemistry, Baylor University / Department of Biology, Baylor University / Department of Chemistry and Biochemistry, Baylor University / Department of Environmental Science, Baylor University / Department of C; L. Aguilar Lazaro, Baylor University / Environmental Science; S. Usenko, Baylor University / Department of Environmental Science, Baylor University / Department of Chemistry and Biochemistry; B.W. Brooks, Baylor University / Environmental Science and Biomedical Studies, Baylor University / Department of Environmental Science. The San Jacinto River Waste Pits was added to the National Priorities list in 2008. Process sludge from paper manufacturing was deposited on the site in the 1960s, and after the site partially subsided into the San Jacinto River in the 1970s and 80s, the surrounding area and biota were contaminated with polychlorinated dibenzo-p-dioxins and -furans. The Houston Ship Channel and lower San Jacinto River also contain noticeable concentrations of PCBs, which are the subject of an ongoing investigation by local, state, and federal regulators and scientists. Fishing advisories were issued in 2001 and 2005 for much of the Houston Ship Channel and upper Galveston Bay estuary to cover concerns regarding consumption of finfish, speckled trout, catfish, and crabs and the associated exposures to PCDD/Fs and PCBs. Prior to cleanup, it will be necessary to characterize the risk to local populations, and a crucial part of that process is to determine the capacity of the contaminants to bioaccumulate in edible fish and shellfish species adjacent to the site. A number of metrics have been devised to accomplish this goal, including biota-sediment accumulation factors, bioaccumulation and -magnification factors, and trophic magnification factors. A unified analysis of bioaccumulation of PCDD/Fs and PCBs at this site will be presented, which incorporates data on contaminant concentrations in water, sediment and biota, with trophic positions of thirteen aquatic species. Comparison of several techniques for calculating these metrics will also be shown. The ultimate outcome of the project is to provide information relevant to the ongoing remedial decision-making for the site while also developing new techniques to extrapolate bioaccumulation between sites with similar contaminant profiles.

**TP107 A unified freshwater-saltwater biotic ligand model to predict olfactory effects of copper to salmonid fishes** J. Meyer, Arcadis U.S., Inc., ARCADIS; D.K. DeForest, Windward Environmental LLC; J. Gorsuch, Copper Development Association. Recent studies have demonstrated short-term exposures to copper (Cu) concentrations as low as 1-2 µg/L in dilute laboratory waters can cause olfactory impairment in freshwater fish, which may limit their ability to migrate or detect predators. Olfactory impairment is of particular concern in the Pacific Northwest because many populations of migratory Pacific salmon and trout (*Oncorhynchus* spp.) are listed as threatened or endangered. However, water chemistry matters in determining lethal and sublethal effects of metals to aquatic organisms. For example, despite effects at low Cu concentrations in dilute laboratory waters, we previously demonstrated that the U.S. Environmental Protection Agency's (USEPA's) BLM-based freshwater aquatic life criteria for Cu protect against olfactory impairment across a wide range of freshwater chemistry; and we developed a biotic ligand model (BLM) to predict olfactory effects of Cu to salmonid fishes in fresh waters. However, no olfactory-impairment studies have been conducted with fish exposed to Cu in salt water, leading to high uncertainty in risk assessments. Moreover, that lack of olfactory studies has impeded development of a saltwater olfactory-parameterized BLM for Cu that would be analogous to the freshwater olfactory-parameterized BLM. To address this situation, we propose a unified BLM to predict olfactory effects of Cu to salmonid fishes across a wide range of fresh and salt waters. Binding constants for Na, Ca, and Mg to olfactory tissue are derived

from neurophysiological studies, and the binding constant for Cu is then optimized by fitting to Cu-olfactory data for freshwater coho salmon (*O. kisutch*). The resulting BL-Na and BL-Cu binding constants are higher than in the previous freshwater olfactory-parameterized BLM, but the BL-Ca and BL-Mg binding constants and the Cu-sensitivity parameter are lower. The unified model predicts that (1) competition of Na and Ca with Cu for binding to olfactory tissue should generally cause the olfactory threshold-effects concentrations for dissolved Cu to be higher in salt water than in fresh water, and (2) USEPA's current acute and chronic saltwater Cu criteria (4.8 and 3.9 µg Cu/L) protect against olfactory impairment across the wide range of dissolved organic carbon (DOC) concentrations that can occur in sea water (~0.5-10 mg DOC/L). However, this unified olfactory BLM awaits empirical testing before it might be accepted for regulatory use.

**TP108 Contaminated sediment affects burrowing behavior of Pacific lamprey (*Lampetra tridentata*) ammocoetes** J.M. Morris, Stratus Consulting; J. Peers, J. Lipton, Stratus Consulting Inc.; C. Schreck, R. Chitwood, J. Unrein, Oregon State University / Oregon Cooperative Fish and Wildlife Research Unit, USGS. Contaminants such as chlorinated hydrocarbons, petroleum-related compounds, metals, and other hazardous substances have been released from various sources into Portland Harbor and have accumulated in sediments. Sediments from specific areas in the harbor are toxic to benthic invertebrates, and benthic organisms and fish in the harbor have accumulated contaminants. Benthic habitat in the harbor may be an important resting and foraging area for larval Pacific lamprey (*Lampetra tridentata*) as they transition to the lower Columbia River and undergo metamorphosis to their adult life stage. Larval lamprey (ammocoetes) in the harbor likely are exposed to a variety of contaminants, and ammocoetes collected from the harbor have been found to accumulate organochlorine compounds to a greater degree than ammocoetes collected from upstream areas. In support of the Portland Harbor Natural Resource Damage Assessment, several laboratory experiments were conducted using field-collected sediments to assess whether contaminated sediments affected ammocoete behavior. Behavioral assays using multiple sediments were conducted to determine if ammocoetes exhibited any preference between reference and contaminated sediments. Additionally, behavioral assays using single sediments were conducted to determine if burrowing behavior metrics such as time to initiate burrowing or time to complete burrowing were different between reference and contaminated sediments. Results indicate that ammocoetes select uncontaminated sediments when given a choice (e.g., 64% reference, 36% contaminated;  $P < 0.01$ ), and that the time to initiate and complete burrowing into contaminated sediment is protracted compared to reference sediments (e.g., ~1-2 minutes longer to initiate and ~8-30 minutes longer to complete burrowing). The utility of conducting burrowing assays for the purposes of assessing sublethal toxicity of sediments will also be discussed.

**TP109 Dissolved Organic Carbon Modulates the Effects of Copper on Olfactory-Mediated Behaviors of Chinook Salmon** P. Stecko, Minnow Environmental; B. Truelsen, Yukon Environment; D. Petkovich, Access Consulting Group; C. Kennedy, Simon Fraser University. The modulation of copper (Cu) effects on olfactory-mediated behaviours by dissolved organic carbon (DOC) and iron (Fe) was examined in juvenile Chinook salmon. Chinook were exposed to several concentrations of Cu, DOC and Fe, alone or in combination in a flow-through system for either 4 days (acute exposure) or 14 days (sub-chronic exposure) and tested for their ability to detect and avoid the odorant L-histidine in an avoidance/preference trough assay. In both acute and sub-chronic exposures, Cu inhibited the ability of fish to detect this amino acid in a concentration-dependent manner, and Cu olfactory inhibition decreased with increasing DOC concentration. In both acute and sub-chronic experiments including DOC, Cu-induced olfactory inhibition decreased in a linear fashion with increasing DOC concentration. The protective effect of DOC on Cu olfactory inhibition was reduced only slightly in the presence of iron, indicating that other metals can potentially affect the modulation of the olfactory inhibition of Cu through competition for DOC binding sites. The results of the present study clearly show DOC amelioration of the effects of Cu to juvenile Chinook salmon olfaction at a behavioral level. These data indicate that DOC concentrations should be considered when evaluating the potential impact of Cu on fish olfaction.

**TP110 Effects of contaminants on the olfactory system of wild fish: relating gene expression, neurophysiology, and behaviour** A. Azizishirazi, Lakehead University / Biology; B.A. Dew, Aquatic Biotechnology and

Ecotoxicology Lab, Lakehead University, Lakehead University, Lakehead University / Department of Biology, Lakehead University / Biology; G. Pyle, Lakehead University / Department of Biology. Chemical communication is essential for vital life processes in aquatic animals such as fish. Exposure to low concentrations of metals has been shown to impair chemical communication in fish. Most of these studies however, were performed under laboratory condition using laboratory model species, while studies using wild animals are rare. To investigate the effects of short term and life-time exposure to metals, wild yellow perch (*Perca flavescens*) from clean and metal contaminated lakes in the industrial area of Sudbury, ON were used. Three levels of biological organization were investigated in this study; gene expression was measured using a novel microarray with olfactory tissue, electro-olfactography (EOG) was used to measure neurophysiological responses of fish, and antipredator assays were used to measure behavioural response to cues. Comparing fish from clean and metal contaminated lakes, which have been exposed to metals for their entire life, demonstrated lower EOG response in fish from metal contaminated lakes. However, there was very little difference in baseline gene expression between fish from each of the lakes. To study the short term effects of metals on the olfactory system of wild fish, perch from a clean lake were exposed to ecologically relevant concentrations of copper or nickel for 24 hours. Results demonstrated that EOG was reduced due to exposure of metals, as well numerous differences in gene expression were measured. Behavioural assays on short term and life time exposures with contaminants will show if the effects seen at the other two levels of biological organization carry through the behavioural level. Taken together, this work is the first to connect the gene expression of wild-caught fish with neurophysiological and behavioural responses.

**TP111 Effects of Copper on Olfactory and Behavioral Responses of Saltwater Fish and the Protectiveness of Biotic Ligand Model-based Aquatic Life Criteria** D.K. DeForest, Windward Environmental LLC; R.W. Gensemer, GEI Consultants, Inc.; J.W. Gorsuch, Copper Development Association Inc.; J.S. Meyer, Arcadis U.S., Inc.; R.C. Santore, HDR|HydroQual, Inc.; B.K. Shephard, US Environmental Protection Agency; J. Zodrow, Arcadis U.S., Inc.. Several studies have evaluated the effects of copper on olfactory function and/or behavior in freshwater fish, including the freshwater life stages of several species of salmonids (e.g., salmon, trout, and whitefish). These studies have demonstrated that olfactory impairment is a more sensitive endpoint than the common acute lethality endpoint. However, the data available to-date indicate that ambient water quality criteria (AWQC) for copper in freshwater are protective against olfactory impairment in fish, particularly when the AWQC were derived using the freshwater biotic ligand model (BLM). With the pending release of draft BLM-based AWQC for copper in saltwater, toxicity data on the olfactory and behavioral effects of copper in saltwater fish species were reviewed. Overall, no olfactory and limited behavioral copper toxicity data were identified for saltwater fish species, and none of the water chemistry data from those studies included dissolved organic carbon (DOC) concentrations, which is one of the most important parameters influencing copper bioavailability. Accordingly, it was necessary to estimate DOC concentrations in the source water and use other approaches for evaluating whether draft saltwater BLM-based copper AWQC are protective against olfactory impairment and behavioral effects. The analyses to-date indicate that the draft AWQC are protective of these endpoints in fish. If proposed research on the effects of copper on olfactory impairment in saltwater life stages of Pacific salmon is conducted, those results will be used to validate a recently developed unified freshwater-saltwater olfactory-based BLM and further evaluate whether saltwater BLM-based AWQC are protective against olfactory impairment in saltwater fish.

**TP112 Impairment of specific olfactory sensory neuron classes by metals: laboratory to field extrapolation** B.A. Dew, Aquatic Biotechnology and Ecotoxicology Lab, Lakehead University, Lakehead University, Lakehead University / Department of Biology, Lakehead University / Biology; A.A. Shirazi, Lakehead University / Biology; G.G. Pyle, Lakehead University / Department of Biology. Olfactory sensory neurons (OSNs), along with support cells, comprise the olfactory epithelium of fish. There are three known OSN cell types; ciliated, microvillous, and crypt cells, all of which can bind chemosensory cues from the water. Once bound, a signal is sent through the neurons to the brain, which may induce a behavioural response. Specific odourant molecules target specific OSN cell types. For example, L-alanine will induce a response specifically in microvillous cells, while taurocholic

acid (TCA) will induce a response specifically in ciliated cells. By using these two odourants with a technique designed to measure the olfactory acuity of a fish, electro-olfactography (EOG), we were able to measure how exposure to nickel or copper specifically affected the function of microvillous or ciliated cells, respectively. The results demonstrated that when fathead minnows were exposed to 20 µg/L copper for 48 h, both ciliated and microvillous cells were impaired, however, at lower concentrations (5 and 10 µg/L), only ciliated cells were impaired. Fathead minnows exposed to three different concentrations of nickel (25, 100, or 500 µg/L) resulted in chemosensory impairment specific to microvillous cells. For comparison, wild yellow perch exposed to 20 µg/L copper for 24 h showed a specific impairment of only ciliated cells, while exposure to 500 µg/L of nickel resulted in specific impairment of microvillous cells. Taken together, these results demonstrate that copper targets ciliated cells in laboratory-reared and wild fish, while nickel targets microvillous cells. Behavioural testing of fish exposed to concentrations of copper and nickel that impair specific OSN classes will demonstrate which classes of OSNs are essential in specific behaviours. By connecting OSN classes with specific behaviours, we can begin to predict behavioural deficits caused by contaminant exposures and improve the ecological relevance of environmental risk assessments of contaminated systems.

**TP113 Regulatory Implications of Chemosensory and Behavioral Effects of Anthropogenic Chemicals to Fish** B.W. Gensemer, GEI Consultants / Ecological Division; D.K. DeForest, Windward Environmental LLC; J. Meyer, Arcadis U.S., Inc., ARCADIS. Regulatory criteria for the protection of aquatic life from exposure to anthropogenic chemicals are based primarily on laboratory toxicity data using test endpoints derived on the basis of survival, growth, and reproduction. These endpoints are generally believed to provide the best representation of overall ecological impacts of chemicals to populations of aquatic organisms in the field. However, some have proposed that aquatic life criteria for copper are insufficiently protective of sublethal chemosensory and behavioral endpoints in salmonid fishes, and therefore regulatory criteria need to be revisited. This talk will review the ecological basis of aquatic life criteria derivation using the more traditional endpoints of survival, growth, and reproduction, and discuss the extent to which other sublethal endpoints may influence how criteria are derived. For copper, we contend that traditionally based aquatic life criteria are adequately protective against chemosensory or behavioral effects in salmonid fishes. In large part this is because other aquatic species (e.g., cladocerans) are more sensitive to copper than are salmonid fishes, but also owing to the influence of water chemistry (i.e., pH, hardness, salinity, and dissolved organic carbon) on the toxicity of copper regardless of whether traditional or non-traditional sublethal endpoints are considered.

**TP114 The effects of anthropogenic compounds on brain chemistry and behavior in fish** A.L. McLeod, Clemson University / Department of Biological Sciences, Clemson University; L. Sweet, Clemson University / Institute of Environmental Toxicology; S.J. Klaine, Clemson University / Institute of Environmental Toxicology (CU-ENTOX), Clemson Institute of Environmental Toxicology / Institute of Environmental Toxicology (ENTOX), Clemson University / Institute of Environmental Toxicology (ENTOX). The sources of anthropogenic compounds in today's society are many and can include personal care products and pharmaceuticals. Some of these compounds are not efficiently removed from the human body or from sewage before they reach streams and rivers, and thus, these compounds are extremely prevalent in today's environment. Once in the ecosystem, they can produce adverse effects on the organisms that live there. Previous work has shown that upon exposure to fluoxetine, a selective serotonin reuptake inhibitor antidepressant, hybrid striped bass (*Morone saxatilis* x *M. chrysops*) brain serotonin levels decreased by 23.7, 28, and 49.1 % upon exposure to 35, 75, and 150 µg/L concentrations for 6 days. Similarly, exposure to venlafaxine, a serotonin and norepinephrine reuptake inhibitor antidepressant, also showed a significant decrease in brain serotonin levels after 6 days of exposure to 50, 250, and 500 µg/L concentrations. These studies correlated depressed brain serotonin levels with behavioral alterations that decrease an organism's ecological fitness. Exposure to bupropion, a norepinephrine and dopamine reuptake inhibitor antidepressant, did not alter behavior as the previous two antidepressants did because it does not target serotonin. This study characterized the effects of copper and chlorpyrifos exposure on brain chemistry and behavior in zebrafish (*Danio rerio*) and the fathead minnow (*P. promelas*) using the standard light/dark anxiety behavioral assay. This study further elucidates the relationship between brain chemistry and

behavior in fish, as well as provides a comparison among the zebrafish and fathead minnow in using the light/dark behavioral assay.

**TP115 Development of an Automated Process for Chemical Safety Assessment within the Toy Industry** J. Rinkevich, P. Beattie, T.G. Osimitz, SciVera LLC. In response to new and revised regulatory requirements like the EU Toy Safety Directive (2009/48/EC), SciVera worked with technical experts from several toy companies in the US and Europe to develop a common approach to verifying the safety of chemicals and materials used in toy products using established hazard, exposure, and risk assessment procedures and then automating that process using software for scalability and reliability. From the initial manual assessment process that was developed, the team configured a web-based software platform to further streamline the process and allow for simultaneous assessments of complex parts made up of multiple chemical substances along with the consideration of preferred alternatives. This automated process has significantly improved the efficiency and cost effectiveness of verifying the safety of toy materials by screening complex products for potential hazard, exposure and risk. Applications of the approach include (1) broad functionality to gather product and material data from widely distributed suppliers of components and raw materials, as well as robust systems and processes for gathering toxicological data on industrial chemicals to support restricted substance list screening and hazard and dose-response assessment; (2) the use of up to 22 human health and environmental hazard endpoints identify chemicals needing exposure assessment and risk characterization; (3) comparisons of hazard and risk profiles across products for safer alternative selection; (4) efficient and secure communication with suppliers, sharing product assessments while protecting confidential business information; (5) establishing priorities for action when assessments indicate the potential for unacceptable risk; and (6) designing-out chemicals that exhibit unacceptable hazard characteristics prior to these substances becoming regulated. The approach provides a robust, transparent and common solution that makes complex hazard, exposure, and risk information accessible to companies for making more informed material decisions.

**TP116 Safer ionic liquids for biomass conversion and processing** N. Gathergood, Dublin City University / School of Chemical Sciences; D. Yang, Dublin City University / School of Chemical Sciences; D.S. Argyropoulos, NC State University / Department of Forest Biomaterials. A goal in our laboratories is to develop new strategies for the transformation of a variety of lignocellulosic resources into value-added materials and chemicals, based on environmentally sustainable and economically viable processing platforms, by utilising ILs unique physical properties. Our understanding of the complete wood dissolution in ILs provides a variety of options for the development of novel homogenous processing platforms aimed at the efficient utilization of lignocellulosic resources on the planet. Enzymatic hydrolysis of cellulose in lignocellulosic materials is one of important parts in our strategy for biorefinery research in Dublin City University (DCU). Biodegradation studies in our group have focussed on ionic liquids containing ester and amide groups. This work was inspired by the development of biodegradable surfactants over the last 50 years. We have reported several examples of readily biodegradable ionic liquids containing esters, and examples containing amides with low antimicrobial activity. There is a balance required between stability of the ionic liquid to the enzyme, biomass and released sugar and the propensity to biodegrade. Our latest progress towards these goals will be presented.

**TP117 Sustainable Anaerobic Adhesive Development** N. Gathergood, Dublin City University / School of Chemical Sciences; A. Porter, Dublin City University / School of Chemistry; D. Birkett, Henkel Adhesive Technologies / ARC Technology Development. Anaerobic adhesives are methacrylate based formulations which remain liquid when exposed to air but harden when confined between metal surfaces. Anaerobic adhesives, originally developed by Loctite Corporation, have found many industrial uses such as locking threaded fasteners, sealing threaded pipe connections, retaining cylindrical machine components and sealing flange joints. This presentation describes product development efforts at Henkel to develop a range of sustainable anaerobic adhesives with excellent health & safety and free of environmental hazard warnings.

**TP118 The use of molecular polarizability as a predictor of the octanol-water partitioning coefficient** C.J. Young, Memorial University of Newfoundland / Department of Chemistry, Memorial University; D. Donaldson, University of Toronto / Department of Chemistry. The octanol-water partitioning coefficient ( $K_{OW}$ ) is a measure of the hydrophobicity of chemical compounds, which is often used to predict and explain their uptake to biota. Experimental measurements of  $K_{OW}$  are difficult, particularly for extremely hydrophobic compounds or novel compounds which are still in the design phase. Thus an attractive alternative is to explore, methods to model or calculate  $K_{OW}$ . Molecular polarizability has recently been demonstrated to be an effective predictor of some important environmental partitioning coefficients. Here, we extend that demonstration to  $K_{OW}$ . Polarizability was calculated using quantum chemical computational techniques for polychlorobenzenes, chloroalkanes, chlorotoluenes, chloronaphthalenes, and chlorinated pesticides. A structure-activity relationship (SAR) was derived from the calculated polarizabilities and measured  $K_{OW}$  values. The resulting SAR was able to predict  $K_{OW}$  for 18 out of 19 compounds not included in the model training set within 20% of their median measured value. Notably, the SAR was able to successfully predict differences in  $K_{OW}$  resulting from different structural isomers. This modeling technique yields a fast determination of  $K_{OW}$ , without the need for experimentation, making it ideal for design of compounds with optimal  $K_{OW}$  values.

**TP119 Biodegradable and low antimicrobial and antifungal toxicity chiral ionic liquids** N. Gathergood, Dublin City University / School of Chemical Sciences; M. Ghavre, Dublin City University / School of Chemical Sciences; M. Spulak, Charles University / Department of Inorganic and Organic Chemistry, Faculty of Pharmacy. Synthesis of enantiomerically pure compounds is of great importance in organic chemistry. Especially in the pharmaceutical industry the synthesis of chiral pharmaceuticals as a single enantiomer by the most economical method is desirable. Hence various methods, including asymmetric synthesis, the chiral pool strategy, classical resolution and either kinetic or dynamic kinetic resolution may be employed to obtain an enantiopure product. Use of chiral ionic liquids as solvents for asymmetric synthesis is a new method to obtain enantiomerically enriched products. ILs are often described as 'green solvents'; mainly due to their negligible vapour pressure. However, assessment of their environmental impact is also a requirement, ILs must not persist in the environment and should be non-toxic or of limited toxicity to the environment and all life within. This is a challenging goal for ionic liquid research today. In our present work a library of CILs has been synthesized from methyl imidazole and readily available chiral starting materials in three steps followed by anion exchange. A range of different anions were incorporated into the CILs in order to modulate their physical / chemical properties. When the CILs were tested against seven strains of bacteria they generally displayed low toxicity, and also exhibited favourable biodegradability in a CO<sub>2</sub> headspace test.

**TP120 Biodegradable, non-bactericidal Ionic liquids** N. Gathergood, Dublin City University / School of Chemical Sciences; R. Gore, Dublin City University / School of Chemical Sciences; B. Quilty, Dublin City University / School of Biotechnology. In our group at DCU ionic liquids studied include imidazolium salts, with alkyl or alkoxy side-chains. The design, preparation and evaluation of antimicrobial toxicity and biodegradability of ionic liquids containing ester or amide groups in the alkyl side chain are presented. Factors improving the biodegradation of surfactants have been successfully applied to ionic liquids. The introduction of a group susceptible to enzymatic hydrolysis was found to greatly improve biodegradation (OECD 301D 'Closed Bottle Test'). The incorporation of ether groups into the ester side-chain significantly reduced the toxicity compared with alkyl ester derivatives. A series of ILs were assessed for toxicity and biodegradability, and screened against 7 bacterial strains, with most of the compounds tested showing low toxicity (MIC values > 20 mg/ml). The CO<sub>2</sub> Headspace test was applied to ionic liquids containing the octylsulphate anion, all of which proved readily biodegradable (>60% over 28 days) or exhibited significant biodegradation (55-59%). Applications of these ionic liquids include hydrogenation, Baylis-Hillman and carbonyl-ene reactions.

**TP121 An Evaluation of the 28 Day Chronic Sediment Test Method with *Leptocheirus plumulosus* to Assess the Toxicity of Pesticides for Registration Purposes** C. Picard, Smithers Viscient / Sediment Ecotoxicology, Smithers Viscient / Senior Research Biologist; M. Bradley, Smithers Viscient / Environmental Toxicology, Smithers Viscient; J.M. Giddings,



Compliance Services International. The original 28 day chronic sediment test method using the estuarine amphipod, *Leptocheirus plumulosus*, was developed and primarily utilized to assess the toxicity of field collected sediment samples. The test method guidance, as well as other researchers, have noted the complexity of the method, along with possible confounding factors and the need for further research. With a recent influx of data call in programs for pesticides and other related materials, the EPA has recommended that testing laboratories use a modified version of the current test method that includes a doubling of the number of replicates for biological observations, as well as pore water measurements that require several additional replicates in each treatment group. In this presentation, we outline some early experiences with this expanded method including control data, logistical issues and areas for further modifications. The expanded test method calls for up to 30 kg of sediment, which needs to be processed prior to use in testing. In addition, approximately 2,800 organisms and 5.0 m<sup>2</sup> of surface area are needed to accommodate the expanded test method. The generation of acceptable results is highly dependent of the sediment utilized in testing and often calls for significant manipulation of natural sediments. Pilot testing with OECD formulated sediment suggested for use in the chronic marine testing by the EPA did not yield acceptable survival. We suggest further research and development to produce a methodology of a reasonable scope that can consistently produce acceptable data for use by regulatory agencies.

**TP122 Are region-specific models better predictors of sediment toxicity than national models?** J. Field, Office of Response & Restoration, NOAA / Assessment and Restoration Division, Office of Response & Restoration, NOAA / Coastal Protection and Restoration Division, NOAA / Office of Response & Restoration; S. Norton, U.S. Environmental Protection Agency / National Center for Environmental Assessment. Using a large nationwide database of matching sediment chemistry and sediment toxicity to marine amphipods, we developed multi-chemical models that predict toxicity from sediment chemistry. The models estimate the probability that a sample will be toxic by using the maximum probability ( $P_{Max}$ ) of results obtained from applying a suite of individual chemical logistic regression models to chemical concentrations measured in a sample. Although overall performance of the nationwide models applied to the nationwide database was good, model performance varied in application to regional subsets of the data. To determine whether model performance could be improved for regional applications by calibrating the models to regional data or developing regional-specific models, we calibrated and compared the performance of 4 multi-chemical models applied to data from the NY/NJ Harbor area: the original set of nationwide models, a broader pool of nationwide models, regional models, and a combination of nationwide and regional models. The final models were developed using a calibration process that substantially improved performance over the uncalibrated models developed using the nationwide dataset. The improvements were achieved by selecting the best performing individual chemical models and eliminating those that performed poorly when applied together. Individual chemical model performance was improved by selecting the best performing model from a pool of possible models that included those developed from region-specific and nationwide datasets, using several different response variables and chemistry normalization approaches. Multi-chemical model performance was improved by eliminating individual chemical models that over-predicted toxicity when selected as the  $P_{Max}$ . Although the best performing  $P_{Max}$  model included both nationwide and region-specific models, the performance of the  $P_{Max}$  model derived using only calibrated nationwide model was nearly as good. This finding suggests that calibrating nationwide models to a regional dataset is more effective in improving model performance than developing regional-specific models. Disclaimer: The views expressed in this presentation are those of the authors and do not necessarily reflect the views or policies of the National Oceanic and Atmospheric Administration or the U.S. Environmental Protection Agency.

**TP123 The Biology of Bioavailability: The Role of Functional Ecology in Exposure Processes** T.S. Bridges, US Army Engineer Research and Development Center, U.S. Army Corps of Engineers / USERDC-WES-EM-D; A. Kennedy, G. Lotufo, J. Steevens, C. Ruiz, J. Coleman, J. Lindsay, U.S. Army Corps of Engineers; G. Matisoff, P. McCall, E. Kaltenberg, Case Western Reserve University; R. Burgess, U.S. Environmental Protection Agency. Contaminant bioaccumulation from benthos to fish, wildlife and humans is the exposure pathway responsible for the risks driving most

cleanup decisions for contaminated sediments sites. Improved approaches for quantifying contaminant bioavailability and exposures within the sediment bed is key to reducing uncertainties in descriptions of risks to benthos and fish that feed on benthos. More accurate exposure characterizations are also necessary to support the challenges of designing, implementing, and monitoring the performance of cost-effective *in situ* remedies for contaminated sediments. Approaches which explicitly consider the functional ecology of the diverse array of species comprising benthos will produce more accurate and certain exposure assessments and predictions for remedial decision-making. Benthic species vary in terms of their position within the sediment column (e.g., shallow vs. deep), their relation to sediment particles and porewater (e.g., tube building vs. free burrowing), and feeding behavior (filter feeding vs. deposit feeding), all of which contribute to differences in contaminant exposure and the notion of contaminant bioavailability. Manipulative experiments with *Leptocheirus plumulosus*, *Eohaustorius estuarius*, *Neanthes arenacodentata*, *Mercenaria mercenaria* were conducted to quantitatively determine the extent of PCB exposure received from contact with overlying water, porewater, and the particulate phase of contaminated sediments. Results demonstrate the role and importance of functional attributes of individual species in determining PCB bioaccumulation into tissues. Results of these exposure experiments with contaminated sediment were supported by studies of particle and solute mixing using conserved tracers. These and follow-on experiments will be used to update the RECOVERY model in order to enable incorporating differences among benthic functional groups into risk assessments and evaluations of alternative remedy designs.

**TP124 Rockin' Round Robin Results from Environment Canada's New Echinoid Sediment Contact Test** L. Taylor, Environment Canada; L. Novak, AquaTox Testing and Consulting Inc.; P. Jackman, C. Buday, R. Scroggins, Environment Canada. Sediments to be disposed at sea under the authority of the Canadian Environmental Protection Act must undergo a waste characterization process required to assess the chemical, physical and/or biological properties of the sediment. The biological toxicity testing methods recommended by Environment Canada presently include a sublethal test on sediment porewater using echinoid fertilization (Environment Canada 1992). Research in recent years has shown that this test can be very sensitive to ammonia, a confounding factor, even in uncontaminated reference site sediments, and so is sometimes unable to distinguish between the toxicity of reference site sediments and contaminated sediments. Also, the porewater test may be identifying highly contaminated sediments as non-toxic as their hydrophobic contaminants are not in porewater (i.e., PAHs, PCBs). One possible solution to these problems is to use an echinoid embryo/larval sediment contact test that is less influenced by confounding factors. This new test has recently undergone method validation within eight different environmental testing laboratories (American and Canadian, government and private). The objective of this round robin was to establish that the new standardized method is sufficiently precise (based on intra- and inter-laboratory precision) and amenable for use as a new EC Reference Method. Several improvements to the draft method were made through the trials and tribulations of novice and experienced investigators within the participating laboratories, in addition to comparing statistically derived endpoints using a reference toxicant, plus field-collected contaminated and reference sediments.

**TP125 Development of a ecotoxicological baseline through the use of biomarkers on the oyster *Crassostrea virginica*** P. Ramirez Romero, Universidad Autonoma Metropolitana / Hidrobiologia, U.A.M. Iztapalapa / Depto. De Hidrobiologia; M.D. Guzman Martinez, L. Elizalde Ramirez, A.L. Espejel Pina, I. Arellano Lopez, J.G. Trejo Ramirez, J.E. Padilla Torres, U.A.M. Iztapalapa / Hidrobiologia; A.D. Nava Montes, Instituto Nacional de Ecologia / CENICA. Through monitoring it is possible to observe changes in environmental quality in space and time, which are useful tools for environmental management. After the BP oil spill of 2010 the Mexican government realized the need for an ecotoxicological baseline to evaluate the possible effects of harmful substances through the use of a variety of biomarkers. The oyster *Crassostrea virginica* was chosen as bioindicator of environmental stress due to its wide distribution and economic importance. The objective of the present work was the implementation of three biomarkers: lysosomal stability, acetyl-cholinesterase activity (AChE) and DNA damage evaluation. Oysters were collected in five sites along the Gulf of Mexico (Pueblo Viejo, Tamiahua, Alvarado, Carmen-Machona and Terminos). In the laboratory the organisms were dissected to obtain hemolymph,

gill and digestive gland. Hemolymph cells were used to evaluate lisosomal stability, this was determined as neutral red dye retention time (NRR). Acetyl-cholinesterase activity was evaluated on the digestive gland through a spectrophotometric technique. And DNA damage was evaluated on gill cells through the comet assay. No statistical differences were found in the NRR results but they presented the following pattern: ALVARADO > TERMINOS > TAMIAHUA > CARMEN-MACHONA > PUEBLO VIEJO. With regards to AchE activity, values varied from  $0.96 \pm 0.005$  U/mg protein in Pueblo Viejo oysters to  $0.047 \pm 0.002$  U/mg protein in Terminos organisms. For the comet assay, different damage categories were identified based on the average longitude of the tail of the comets. All cells of all sites showed damaged, however no statistical difference among sites was found. The highest tail length average was detected in Tamiahua organisms and the smallest in oysters from Alvarado. Results in general presented high variability even though organisms were homogenous in size and their collection was done in the same season. It is necessary to continue this work with sampling events in the dry as well as the rainy season. Also, bioassays in which the oysters are exposed to crude oil are necessary to know the variability of the biomarkers and therefore to establish the correct sample size in case of an oil spill event.

**TP126 Saltwater Pb speciation and toxicity for *Mytilus* embryo tests in the presence of various sources and concentrations of organic matter** R. Diamond, Wilfrid Laurier University / Chemistry; S. Smith, Wilfrid Laurier University / Department of Chemistry, Wilfrid Laurier University / Chemistry; S. Nadella, McMaster University; A. Bianchini, Universidade Federal do Rio Grande / Instituto de Ciências Biológicas; C. Wood, McMaster University / Department of Biology. Developing quantitative bioavailability models to assist in the establishment of environmental regulation, as well as for risk assessment purposes, require specific understanding of the links between metal speciation and toxicity. In particular, dissolved organic carbon (DOC) can potentially have an influence on controlling Pb speciation and reducing bioavailability in aquatic media. In the present study, Pb speciation was determined by both fluorescence quenching and voltammetry for two DOC sources at low (2) and high (12 mg C/L) concentrations in sea water. For these same DOC samples, Pb EC50 values were determined for early life-stage development of the blue mussel *Mytilus galloprovincialis* and *Mytilus trossulus*. In the absence of DOC, the EC50 value determined for Pb with *Mytilus galloprovincialis* was 304 (173-453) nM. In the presence of Nordic Reservoir organic matter at 2 mg C/L, EC50 value was 738 (680-796) nM while DOC at 12 mg C/L was 757 (680-830) nM. Thus, DOC is observed to be protective but not in a dose dependent manner. Similar results were observed for *M. trossulus* and also for other sources of DOC. Typical bioavailability models would predict if low DOC were protective, higher DOC should be more protective. Binding capacity for Pb determined by fluorescence quenching was measured at 6.35 nM in low DOC solution and 32.3 nM in high DOC solution. These results are inconsistent with the EC50 determinations. Binding capacity, as determined by fluorescence quenching method, is dose dependent and the quantitative values are far too low to account for the observed degree of protection. Voltammetry was tested as an alternate technique to estimate binding capacity. Using anodic stripping voltammetry, binding capacities of 320 (206 – 434) nM and 347 (279-415) nM were observed for the low and high DOC samples, respectively. These results are consistent quantitatively with the degree of protection and the lack of dose dependence observed. One possible explanation of these observations is salt-induced colloid formation reducing available Pb binding sites as DOC associates with DOC at higher concentrations in the presence of salts. The implication is that future bioavailability models may have to include non-linear binding capacity estimation methods, but more research is needed to test the salt-induced colloid hypothesis. (NSERC Discovery, CRD Program, CRC program, ILZRO, IZA, ICA, CDA, NiPERA, Teck Resources, Vale Canada, Xstrata Zinc).

**TP127 Recreating the seawater mixture composition of HOCs in toxicity tests with *Artemia franciscana* by passive dosing** E. Rojo-Nieto, University of Cadiz / Department of Environmental Technologies; K. Smith, Aarhus University / Department of Environmental Science; J. Perales, University of Cadiz / Department of Environmental Technologies; P. Mayer, Aarhus University / Department of Environmental Science, National Environmental Research Inst. / Environmental Chemistry & Microbiology. The toxicity testing of hydrophobic organic compounds (HOCs) in aquatic media is generally challenging, and this is even more problematic for mixtures. The hydrophobic properties of these compounds make them difficult

to dissolve, and subsequently to maintain constant exposure concentrations. Evaporative and sorptive losses are highly compound-specific, which can alter not only total concentrations, but also the proportions between the compounds in the mixture. Therefore, the general aim of this study was to explore the potential of passive dosing for testing the toxicity of a PAH mixture that recreates the mixture composition found in seawater from a coastal area of Spain, the Bay of Algeciras. First, solvent spiking and passive dosing were compared for their suitability to determine the acute toxicity to *Artemia franciscana* nauplii of several PAHs at their respective solubility limits. Second, passive dosing was applied to recreate the seawater mixture composition of PAHs measured in a Spanish monitoring program, to test the toxicity of this mixture at different levels. HPLC analysis was used to confirm the reproducibility of the dissolved exposure concentrations for the individual PAHs and mixtures. This study shows that passive dosing has some important benefits in comparison with solvent spiking for testing HOCs in aquatic media. These include maintaining constant exposure concentrations, leading to higher reproducibility and a relative increase in toxicity. Passive dosing is also able to faithfully reproduce real mixtures of HOCs such as PAHs, in toxicity tests, reproducing both the levels and proportions of the different compounds. This provides a useful approach for studying the toxicity of environmental mixtures of HOCs, both with a view to investigating their toxicity but also for determining safety factors before such mixtures result in detrimental effects.

**TP128 Using a Novel Sediment Exposure to Determine the Effects of Bifenthrin on Estuarine Benthic Communities** K.T.Ho, U.S. EPA / Atlantic Ecology Division; L.M. Portis, U.S. EPA; A. Chariton, CSIRO / Land and Water; D. Proestou, US Environmental Protection Agency / Atlantic Ecology Division; M. Pelletier, National Health and Environmental Effects Research Laboratory, US EPA / Dept: ORD NHEERL AED, U.S. EPA / Dept: ORD NHEERL AED; M.G. Cantwell, / Atlantic Ecology Division; R.M. Burgess, U.S. EPA / Atlantic Ecology Division, National Health and Environmental Effects Research Laboratory, US EPA, U.S. EPA, U.S. Environmental Protection Agency / Ord/Nheerl-Atlantic Ecology Div, U.S. EPA / Ord/Nheerl-Atlantic Ecology Div; M.M. Perron, NRC/EPA; S. Simpson, CSIRO Land and Water / Centre for Environmental Contaminants Research; J. Baguley, V. Menons, University of Nevada. Bifenthrin is a widely used pyrethroid insecticide that affects animal sodium ion channels in the peripheral and central nervous system of target and non-target species, eventually causing paralysis. It is a suspected human carcinogen and has been banned for use in the European Union; however in the United States over 600 products containing bifenthrin still exist. Bifenthrin is moderately toxic to mammals (LD50 parts per million) but very toxic to fish and aquatic organisms (LC50s parts per trillion). It enters estuarine environments primarily via agricultural and residential use, and, because it is a hydrophobic compound (log Kow ~ 6), it has the potential to accumulate in estuarine sediments. The objective of our research was to determine the effect of bifenthrin on intact estuarine benthic communities. We adapted a novel exposure method which brings intact sediment cores into the laboratory and then exposes the benthic community by addition of toxicant-spiked sediments to the sediment surface. Macro- and meiobenthic communities were analyzed for differences after a two-week exposure to the following treatments: 1) field control – no added sediment, 2) laboratory control – reference sediment added, 3) low bifenthrin concentration (8.5 mg/kg dry), and 4) high (68 mg/kg dry) bifenthrin concentration. Results from the bifenthrin sediment additions indicated a significant difference ( $p < 0.05$ ) in macrofaunal taxa community composition between the controls and both bifenthrin treatments as well as between the low and high bifenthrin treatments. Meiofauna data indicated a significant difference in community composition between the controls and the high bifenthrin exposure. We also noted a difference in the field control relative to the laboratory control, most likely due to a smothering effect of the added sediment. These data suggest that at elevated concentrations, bifenthrin can disrupt benthic communities while also indicating that the laboratory exposure system does not completely emulate exposures in the field.

**TP129 The Regression Can See What You Can't: Identifying Toxicants in Complex Effluents** K. Pilgrim, Barr Engineering Company. Techniques employed and guidance available to identify the cause(s) of industrial or municipal whole effluent toxicity appear to rely upon the assumption that effluents are static entities. However, effluents, particularly industrial effluents, are typically highly variable and complex with potentially multiple

toxics and constituents that can enhance or reduce toxicity. This is particularly true for effluents with elevated dissolved salts and variable influents that may change by the day of the week, season, or even climate. High frequency testing and logistic regression is one approach to address this inherent complexity and variability. We have typically paired whole effluent toxicity testing with a set of chemical analysis, however, the logistic regression approach can also incorporate selective chemical removal approaches such as chemical precipitation, filtration, and ion exchange on a high frequency basis if there is an initial hypothesis of what the toxicant may be. This approach has been applied to several industrial effluents and the results will be provided in this presentation. Particular emphasis will be placed on the following toxicants or toxic mechanisms: pH rise during whole effluent toxicity testing, bicarbonate, ammonia, potassium, and sulfate. The presentation will also show how a toxicant identified using logistic regression can be confirmed as the toxicant or one of the toxicants responsible for a whole effluent toxicity testing failure.

**TP130 Biodegradability tests in seawater for non-aqueous fluids – Comparison of methods** V. Luiz, L. Kraus, LABTOX – Laboratório de Análise Ambiental; J. Salanitro, Dept. of Civil and Environmental Engineering – University of Houston; A. Marroquim, Shell-Brazil. To regulate the use of non-aqueous drilling fluids (NAF) in offshore drilling activities in Brazil, the Brazilian Environmental Agency (IBAMA) requires that biodegradation tests be run with the base oils used. Among the marine aerobic biodegradation test methods accepted by IBAMA are the OECD306D Closed Bottle BOD – Biodegradability in Seawater (1992) and the Two Phase Closed Bottle test (BODIS Test – ISO10.708:1997). The first one is used for water soluble products and the last one is used for testing poorly soluble compounds.

The aim of this study is to describe laboratory studies that have compared a modified OECD306 method with the Marine BODIS test, identifying their critical aspects and suitability to evaluate the aerobic biodegradation of base oils. The tests were conducted with three chemicals with known biodegradation: ethylolate, an ester easily biodegradable; 1-hexadecene, an  $\alpha$ -olefin with intermediate biodegradation; and squalane, a branched paraffin poorly biodegradable. Both tests were incubated at 20°C for 28 days. They used natural seawater with the addition of nutrients and naturally-occurring seawater microbes as source of bacteria. The BODIS tests used flasks filled with medium to about 2/3 leaving a headspace of air and were kept shaking continuously. The tests were run in triplicates that were maintained throughout the experiment by weekly measurements of the oxygen consumption, after which the flasks were aerated for 15 min and incubated again. The OECD 306 test, on the other hand, used flasks filled to the top that were kept without agitation and the replicates were disposed after the weekly measurements of the oxygen consumption. In this study, however, this method was adapted with the following modifications: a) the initial dissolved oxygen concentration was increased to 28-30mg/L using oxygen gas sparging; b) the test chemical was applied to inert filter disks at concentrations of 4 mg/L seawater; c) the flasks were kept under continuous shaking; and d) marine sediment was added (100 mg dry wt./L seawater) to increase the number and diversity of microbial species. Both tests have produced results that show ethylolate as the most easily biodegradable of the three chemicals and squalane as the least biodegradable under aerobic conditions. The variability of the results obtained with the modified OECD306 was larger than that of the Marine BODIS test results.

**TP131 Flow-through ISE Determination of free Cu in sea water and influence of DOC source on free Cu and toxicity to *Brachionus plicatilis*** T. Tait, D.S. Smith, Wilfrid Laurier University / Department of Chemistry.

Copper is a trace element that is essential for proper functioning of plants, animals and microorganisms; however, at increased concentrations Cu can prove toxic. Anthropogenic release of copper has made this metal a common contaminant in marine systems due to its use as a biocide in antifouling paints and coatings. Copper can exist in many different forms in aquatic environments and factors within these environments can affect the speciation of copper. Copper in organic and inorganic complexes is unavailable to interact with organisms to cause toxicity. As such, free copper is often used as an indicator for toxicity since it is the species most available to be taken up by an organism. Thus, the determination of copper speciation is important in understanding the bioavailability of copper and its potential to cause toxicity to marine organisms. Free ionic copper was measured using a flow-through ion selective electrode (ISE). Four different marine samples were collected from various locations and analyzed during a

fixed pH copper titration using a published external standard calibration ISE method. Free cupric determinations in the range  $10^{-12}$  to  $10^{-7}$  mol/L were consistent with published literature but replicate measures showed up to two orders of magnitude variability. To improve reproducibility an internal calibration method was developed. The free copper measured using the improved method showed the same trends as the external calibration data but reproducibility increased to an order of magnitude or better. For example, in one sample the external calibration yielded a concentration range from  $10^{-10.5}$  to  $10^{-7.5}$  mol/L and this was reduced to  $10^{-9.9}$  to  $10^{-9.5}$  mol/L using the internal calibration method. The new method was validated using artificial seawater with added tryptophan as the model ligand. The free Cu was modelled using NIST binding constants and compared well to measured values. As such, this new method will be integrated with toxicity assays to simultaneously measure free copper while determining the effect of DOC source on Cu toxicity to the rotifer *Brachionus plicatilis*. This ability to reliably measure free copper is significant for predicting and measuring toxicity upon copper exposure.

**TP132 Influence of seawater-derived organic matter on acute Zn toxicity in the copepod *Acartia tonsa* in a wide range of salinities** T.R. Avila, Universidade Federal do Rio Grande – FURG / Instituto de Ciências Biológicas – ICB; B.B. Furci, A.d. Machado, A. Bianchini, Universidade Federal do Rio Grande – FURG / Instituto de Ciências Biológicas.

As an important component of estuarine and marine food webs worldwide, the copepod *Acartia tonsa* was used to test the combined effect of Zn and seawater-derived dissolved organic matter (DOM: 1, 2.5 and 10 mg C/L) in a wide range of water salinities (5, 15 and 30 ppt). Acute (48h) toxicity tests were run in duplicate (n=10 per replica). Copepods were incubated in 50-mL glass beakers filled with artificial sea water at the desired experimental salinity. The isolated effect of Zn and DOM were also tested at the same experimental conditions. The respective controls (no zinc or DOM addition in the water) were also performed in each experimental salinity. The experimental media were completely renewed after 24h of exposure. Copepod survival was monitored at the end of the 48-h exposure period. Lack of movement for 15 s was considered as death criterion. Accumulated mortality data were used to calculate the effect concentration for 50% of the individuals tested (EC50) for each treatment. In the absence of zinc, DOM at 10 mg C/L was shown to be toxic to *A. tonsa* in salinity 5 ppt. Therefore, the combined effect of Zn and DOM at salinity 5 ppt was tested using 2.5 mg C/L. The Zn EC50 values (and corresponding 95% CI) in the absence and in the presence of DOM corresponded to 229 (200-263)  $\mu\text{g Zn/L}$  and 356 (335-379)  $\mu\text{g Zn/L}$ , respectively. At salinity 15 ppt, no significant difference was observed between the Zn EC50 values in the absence and in the presence of 1 or 2.5 mg C/L. However, Zn EC50 value was significantly higher in the presence of 10 mg C/L [2,480 (2,019-3,046)  $\mu\text{g Zn/L}$ ] than in the absence of DOM [840 (689-1024)  $\mu\text{g Zn/L}$ ]. At salinity 30 ppt, no significant difference was observed between the Zn EC50 values in the absence [904 (785-1042)  $\mu\text{g Zn/L}$ ] and in the presence of 1, 2.5 or 10 mg C/L [817 (684-977)  $\mu\text{g Zn/L}$ ]. Results show a clear protective effect of increasing salinity against the acute Zn toxicity from salinity 5 to 15 ppt, when a threshold is apparently achieved near the isosmotic point (between 15 and 30 ppt). They also indicated a marked protective effect of DOM against this toxicity in estuarine waters. Based on these findings, Zn accumulation experiments were run at the EC50 values to evaluate if water salinity and DOM are influencing the whole-body lethal accumulation (LA50) values at the different experimental conditions. [Support: International Zinc Association (USA), IDRC (Canada), CNPq (Brazil) and CAPES (Brazil)]

**TP133 Effect of salinity and natural organic matter on the toxicity of Cu to *Americamysis bahia*** R. Nasir, J. Cunningham, S. Smith, Wilfrid Laurier U.; J. McGeer, Wilfrid Laurier University / Department of Biology. The understanding of the potential impact of Cu in estuarine waters is complicated by the effect salinity can have on geochemical speciation as well as the physiology of organisms. Additionally the protective capacity of dissolved organic carbon (DOC) on Cu toxicity, which has been characterized in both fresh & marine waters, is not well understood at intermediate salinities. This lack of knowledge represents an important uncertainty in the development of water quality criteria and guidelines for Cu in estuarine conditions. The goals of this research is to develop and improved understanding of the acute toxicity of Cu to *Americamysis bahia* across a salinity gradient and in the presence of natural organic matter (NOM). Cultures were established at 15 & 25 ppt and 96 h toxicity tests were done at 25°C with offspring aged 2-4



d. Test solutions were made 24 h prior to initiating tests and solutions were renewed at 48h. Four test series were developed: 1) the effect of salinity (5 – 40 ppt) on Cu toxicity, 2) the effect of culture acclimation salinity (15 or 25 ppt) on Cu toxicity at different salinities, 3) the protective effect of DOC on Cu toxicity at different salinities, and 4) the variability among NOM sources on its protective capacity. The 96 h LC50 for Cu increased from 68 (95% CI 46 – 99)  $\mu\text{g Cu/L}$  at a salinity of 5 ppt to an average of 277  $\mu\text{g Cu/L}$  at salinities between 15 and 30 ppt. However at 35 ppt LC50 was reduced 195 (103-243)  $\mu\text{g Cu/L}$ . When comparing responses to Cu at 15 and 25 ppt, LC50 values were similar for offspring from cultures irrespective of the acclimation salinity (15 or 25 ppt). A concentration dependent protective effect of DOC was observed with added of 2, 4 and 10 mg C/L, at 15 ppt up to a 3 fold reduction in toxicity. DOC mitigation of Cu toxicity was tested at salinities of 15, 25 and 35 ppt and the degree of protection generally declined as salinity increased. NOM from freshwater sources that discharge into estuaries were collected just upstream of the saline mixing zone by reverse osmosis concentration. Sources were in New Brunswick (Kouchibouguac National Park) and Prince Edward Island (Southampton Wildlife Management Area & North Lake). This study is contributing to the development of a site specific approaches for setting water quality criteria and is funded through a NSERC CRD grant with ICA, CDA, ILZRO, IZA, Teck, Xstrata Zinc.

**TP134 Removal rates of TNT and RDX in the marine environment: the influence of sediments** R.W. Smith, P. Vlahos, C. Tobias, M. Ballentine, T. Ariyaratna, C. Cooper, University of Connecticut at Avery Point / Department of Marine Sciences. Coastal military activity has resulted in marine sites containing unexploded ordnances (UXOs) and dissolved and particulate munitions compounds. UXOs enter the marine environment from the use of explosive weapons (a percentage of which are faulty) in wars or training exercises, as well as through worldwide disposal at sea. Seawater rusts the iron casings resulting in the release of solubilized munition compounds into the water. 2,4,6-trinitrotoluene (TNT) and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) are two of the most commonly used high-explosive compounds, occurring together as a mix in comp B and other formulations. TNT and RDX are carcinogenic, mutagenic, and toxigenic, and pose health risks to biology including humans. These compounds have also been determined to be toxic to marine organisms dissolved in seawater or adsorbed on marine sediments. In order to determine how toxicity end-points measured in the lab translate to negative impacts in actual marine systems, it is necessary to evaluate the removal rates from seawater (and therefore the residence time of the compounds) via sediment adsorption and biotic and abiotic transformation and remineralization, as it has a direct impact on the exposure time of organisms. In this study, we model the behavior of RDX and TNT in the coastal marine environment, both in the water column and at the sediment/water interface, in a series of mesocosms containing recirculating natural seawater and coastal sediments. Two sediment types were used, one finer-grained, silty sediment and one coarser-grained, sandy sediment. In all cases, removal rates were the slowest (or sometimes negligible) in the tanks containing no sediment than in the tanks containing sediment. Additionally, removal rates were significantly amplified in finer-grained sediment. Calculated half-lives in our study were faster than reported rates in the literature, ranging from 1.3 – 3.8 days for TNT, 12-72 days for RDX, 2 – 28 days for 2-aminodinitrotoluene (2a-DNT, TNT breakdown product), and 8 – 41 days for 4-aminodinitrotoluene (4a-DNT). The discrepancies in the calculated process descriptors among studies available in the literature are potentially related to temperature as well variability in the natural microbial systems in the seawater and sediments utilized in the study capable of breaking down explosives compounds.

**TP135 Modeling the role of extracellular polysaccharides secretion as a mechanism of mercury tolerance in deep-sea hydrothermal vent bacteria using *E. coli*** K.A. Cruz, Rutgers University & UMDNJ / Joint Graduate Program in Toxicology, Department of Biochemistry & Microbiology; C. Vetriani, Rutgers University / Institute of Marine and Coastal Sciences; T. Barkay, Rutgers University / Department of Biochemistry and Microbiology. Deep-sea hydrothermal vents are a natural source of mercury, and organisms living there have evolved mechanisms to cope with elevated levels of mercury. Bacteria transform mercury between its oxidation states and the organic and inorganic forms resulting in movement throughout the environment and alterations in mercury bioavailability. Chemosynthetic bacteria isolated from the vents grow in high levels of mercury although they do not detoxify

it by conversion to the volatile elemental form, the classic mechanism of microbial mercury resistance. It is known that vent bacteria produce extracellular polysaccharides (EPS) helping their survival at the vent site. EPS has also been shown to sequester heavy metals extracellularly. Therefore, the role of EPS production on mercury resistance was studied with the well-defined model organism *Escherichia coli*. Methods: The *E. coli* strains selected do not detoxify mercury by its reduction. The wild type (strain ZK2686) produces the EPS, colanic acid, while the mutant (strain ZK2687) is defective in EPS production. The two strains were grown in a minimal media without sugar and in the presence of up to 5  $\mu\text{M HgCl}_2$ . Bacterial growth was determined by optical density at 660 nm over time. During the course of growth samples were taken to measure EPS production by the phenol-sulfuric acid assay for sugar. Results and Conclusions: The *E. coli* mutant produced less EPS per cell than the wild type and was less tolerant to mercury than the wild type strain. This result is a proof-of-principle that EPS production may facilitate mercury tolerance by sequestering mercury outside of the cell preventing intracellular damage. Since deep-sea vent bacteria are known to produce EPS, it is plausible that the secretion of EPS is part of the mechanism of mercury tolerance in these organisms. This hypothesis is currently being tested with the mercury resistant deep-sea vent bacterial strains.

**TP136 Effects of water-based drill cutting on calcareous algae of a nearshore rhodolith bed within a protected area at Campos Basin, Brazil** M. Reynier, LABTOX – Laboratório de Análise Ambiental Ltda; F. Tamega, Instituto Biodiversidade Marinha; S. Alves, LABTOX – Laboratório de Análise Ambiental Ltda; M. Figueiredo, Instituto de Pesquisa Jardim Botânico do Rio de Janeiro. Rhodolith beds are marine communities dominated and built by free-living calcareous algae worldwide distributed that largely cover Brazilian continental shelf and are often exposed to high turbidity at nearshore. These beds built upon soft sediments create critical habitats for benthic fauna. This study presents an assessment of water-based drill cuttings, barite, bentonite and natural sediments on calcareous algae in a static renewed-test. The rhodolith calcareous algae *Lithothamnion* sp. was collected from the shallow beach of João Fernandes, Búzios, RJ. Samples were taken by scuba diving and then transported to the laboratory where they were kept under same environmental conditions of irradiance ( $100\mu\text{mol.m}^{-2}.\text{s}^{-1}$ ) and temperature (22°C). One rhodolith sample of standard volume ( $5\text{cm}^3$ ) was used in each replicated culture dish ( $n=6$ ). In each treatment dish it was added 50g of either natural sediments, drill cuttings, barite or bentonite, in order to have a thin and scattered layer smothering rhodoliths. Once a month these samples were retrieved and incubated in 0.45  $\mu\text{m}$  filtered seawater in DBO bottles (300ml). One sample was kept in complete darkness and an initial bottle filled only with seawater was included to discount for plankton oxygen consumption and production, respectively. Dissolved oxygen readings were measured after 4 hours by using a sensor with a stirrer linked to an oxymeter. After 90 days in culture, each sample was oven-dried (60°C) to correct oxygen readings to its dry weight (g). The net photosynthesis and respiration rates were calculated from Thomas's formulas (1988). One-way ANOVA and Tukey tests were performed to detected differences among treatments and their controls (seawater alone). Significant differences were detected for drill cuttings and barite which showed lower net photosynthesis after two months (ANOVA,  $F=5.03$ ;  $P\leq 0.05$ ) and for drill cuttings, barite and natural sediments after three months (ANOVA,  $F=3.07$ ;  $P\leq 0.03$ ). The main impact of discharged water-based cuttings was assumed to be mainly physical instead of chemical effect. It depended on the time of exposure. In this experiment results suggested that effects might be due to partial light attenuation and partial restriction on gas exchange due to rhodoliths being partially covered by sediments. These results serve as a reference test for deep-water calcareous algae environments. This is part of a long term project that aims to test physical and chemical effects of drill cuttings and different sources of sediments.

**TP137 Marine debris in the Portuguese coast: An extended assessment of size distribution, uses and contaminants in stranded plastics** J.C. Antunes, J. Frias, Faculty of Science and Technology / Department of Environmental Sciences and Engineering; P. Sobral, Faculty of Science and Technology; C. Micaelo, A. Ferreira, IPIMAR. Nowadays microplastic marine debris are an environmental issue for marine communities and oceans, due to their capacity to adsorb persistent organic pollutants and similarity in size to marine organisms food items. In order to understand marine pollution by these particles, stranded marine debris were collected in ten selected beaches along the coast of Portugal. Samples were analyzed by type of

material and plastics were categorized according to use and composition. All plastics were measured according to three different size classes: 1 to 10 mm, 11 to 25 mm and larger than 25 mm. Approximately 13 kg of marine debris and 31674 items were collected in 154 m<sup>2</sup> of sampled sand (8302 items m<sup>-2</sup>), where 98% were plastic (31027 items, 12.19 kg), particularly resin pellets (52.5%, 16304 items, 368 g) and plastic fragments sized between 1 to 10 mm (85.7%, 26592 items, 613.8g). Microplastics (< 5 mm) represented 49% of marine debris collected of which 52.5 % were resin pellets. Stranded pellets were sorted in four classes: white, for virgin resin pellets, aged, for yellow-brownish pellets indicating long term exposure in the oceans, coloured for all the coloured pellets, (except black) and black for black pellets. Polycyclic aromatic hydrocarbons (PAH), polychlorinated biphenyls (PCB) and DDT were analyzed through gas chromatography mass spectrometry (GC-MS). The highest concentrations of PAH were found at Sines beach, particularly in aged and black pellets (11860 ng.g<sup>-1</sup> and 44800 ng.g<sup>-1</sup>, respectively). Phenanthrene, pyrene and fluoranthene were the compounds with higher concentrations in this site (18374, 9339 and 5612 ng.g<sup>-1</sup>). Higher concentrations of PCB were found for congeners 138, 149, 153 and 187 (35.52 ng.g<sup>-1</sup> to 46.81 ng.g<sup>-1</sup>). Aged pellets were the class with higher PCB contamination, especially in Matosinhos beach. The higher concentration of DDT is expressed in aged pellets results for Matosinhos beach (41 ng.g<sup>-1</sup>). This project is an on-going work-in-progress that aims to better understand the correlation between persistent organic pollutants adsorption and plastic degradation.

**TP138 Considering hyporheic exchange in stream projects: streambed contaminations** D. Tonina, University of Idaho / Civil Engineering. The availability of healthy riverine habitat is a crucial element for viable aquatic population and for supporting benthic and hyporheic ecotone. The last identifies all those organisms that permanently or for part of their life stages dwell in the streambed sediment. This is a rich ecotone, which depends on the physical properties of both surface and subsurface water. Consequently, river works may alter these surface and subsurface habitats. Whereas contaminant impact on surface water is well recognized a less known risk is the contamination of streambed sediment, which could potentially extend both laterally and vertically for several meters. Stream water continuously exchanges between river and streambed material such that solutes, particulates and pollutants are transported within the sediment where they may be temporally or permanently stored and/or transformed. This advective transport is known as hyporheic exchange. It is primarily driven by near-bed head gradients, stemming from the interaction between stream flow and stream morphology. Biophysical interactions associated with salmonid spawning also induce localized hyporheic flows, which provide surface oxygen-rich waters to incubating embryos. Salmonids bury their eggs in streambed gravels for incubation in nests within the near-surface hyporheic zone, called redds. Redd construction modifies channel topography, creating a pit and a hump, and winnows fine grains from the streambed. This process results in relatively coarser and more porous sediment, with higher hydraulic conductivity than the undisturbed bed material and a shape that enhances exchange. Consequently, hyporheic exchange induced by redds will also direct toxins and pollutants that are present in the streams into the streambed sediment and into egg pockets. Oil spills in streams may diffuse within the streambed sediment affecting fish population and streambed ecotone. Here, we introduce the effects of streambed topography on hyporheic fluxes at the reach scale. Then we examine the effects of spawning activity on hyporheic exchange at the local scale. Results show that salmon nests significantly alter local river hydraulics and local patterns of upwelling and downwelling, enhancing hyporheic exchange through the pool tail and egg pocket, thereby potentially enhancing offspring survival. Consequently, these benefits may make the eggs vulnerable to oils spills and increasing the risk of streambed contaminations.

**TP139 NRDA Pre-assessment Phase Case Study: Yellowstone River Oil Spill** M. Carney, K. Ritter, Stratus Consulting Inc.. The Yellowstone River is one of the largest un-dammed rivers in the US. As such, this free-flowing, braided river system represents a uniquely important ecosystem with key aquatic wildlife habitat features including productive backwater areas and abundant large woody debris piles. On July 1-2, 2011 an estimated 64,000 gallons of crude oil spilled into the Yellowstone River near Laurel, Montana from a 12-inch pipeline break. The spill occurred during river flood stage and oil was distributed throughout the inundated floodplain, extending as far as approximately 65 miles downstream of the point of discharge. This

presentation provides a description of response actions to recover oil and wildlife and natural resource damage assessment activities conducted by Trustees to evaluate potential injuries to natural resources associated with removal actions and residual oil in the environment.

**TP140 Forensic Differentiation of Diluted Bitumen Products from Pyrogenic, Biogenic and other Petrogenic Background Sources – The Kalamazoo River Oil Spill** B. Hollebone, Environment Canada / Emergencies Science & Technology; G. Douglas, NewFields Environmental Forensics Practice; C. Yang, Environment Canada; Z. Wang, Environment Canada / Emergencies Science and Technology. Following the break of an Enbridge pipeline near Marshall MI, in late July 2010, 19,000 barrels (3.1 ML) of petroleum products were released into Talmadge Creek and the Kalamazoo River. Enbridge identified the products as a combination of Western Canada Select and Cold Lake diluted bitumens (dilbit). A 40-mile (65 km) stretch of river was affected, from the point of release to Morrow Lake Dam. Much of the dilbit sank to the bottom of the river as small, weathered globules of oil and oily sediment, following loss of lighter hydrocarbon fractions and by mixture with river sediments. The spill of diluted bitumen products into the Kalamazoo River presented a series of challenges to identification and quantification of the spilled oil product from pre-existing background levels of total petroleum hydrocarbons. An active ecosystem along the river results in biogenic background in total petroleum hydrocarbon (TPH) and individual chemical background levels. Natural and anthropogenic combustion sources have left the river with high background levels of EPA Priority polycyclic aromatic hydrocarbon (PAH) levels. Historical industrial and recreational uses of the river have also left traces of petroleum in the river sediments. The present work discusses the differentiability of the dilbit chemical distributions from the background signatures of biogenic, pyrogenic and other petrogenic inputs. The ranges of variation in biogenic, pyrogenic and other petrogenic sources from the literature are examined and compared to the variability shown in sheen, free globule and core samples collected at the spill site. Specific metrics are discussed in terms of identifying the types on inputs for sediments cores by use of forensic diagnostic ratios and other indices. These results will be used the natural resource damage assessment (NRDA), to assess mass balance of the oil remaining in the river and to develop clean-up endpoints for spill response and remediation.

**TP141 Analysis of PFOA in Home Grown Produce, Untreated Irrigation Water, Soil, Beef Tissue and Fodder Near a Fluoropolymer Manufacturing Facility** A.S Hartten, DuPont / Corporate Remediation Group; T. Bingman, DuPont Company / Corporate Remediation Group. Multi-media sampling was conducted within a five-mile radius of a fluoropolymer manufacturing facility (Site) in West Virginia to evaluate the presence of perfluorooctanoic acid (PFOA) in environmental media. A total of 29 residential locations near the Site and one background location were selected for sample collection. Environmental media sampled included five produce types (tomatoes, peppers, green beans, cucumbers and potatoes), untreated irrigation water (if used for growing the produce), and the soil in which the produce was grown. In addition, beef tissue was sampled at one location, as were untreated water sources and the fodder consumed by the cattle. PFOA was not detected in 106 of the 108 total produce samples analyzed. It was measured at concentrations of 0.89 ug/kg and 0.98 ug/kg in two green bean samples. Concentrations of PFOA in the eight untreated irrigation waters and in the 30 soils ranged from non-detect (< 0.0034 ug/L) to 10 ug/L and from non-quantifiable (< 1.5 ug/kg) to 33 ug/kg, respectively. PFOA was not detected in the beef tissue at a detection limit of 0.43 ug/kg. PFOA measured in the four cattle waters ranged from 9.2 ug/L to 19 ug/L. PFOA was detected at a concentration of 66 ug/kg in the cattle fodder. These data indicate no or minimal uptake of PFOA from untreated water and soil to the produce and from untreated water and fodder to beef tissue. These data also suggest that ingestion of locally grown produce and beef are unlikely to represent significant contributions of PFOA to human exposure.

**TP142 Assessment of Best Management Practice Effects on Metolachlor Mitigation in an Agricultural Watershed** R.E Lizotte, USDA Agricultural Research Service / National Sedimentation Laboratory, USDA-ARS National Sedimentation Lab / Water Quality and Ecology Research Unit, USDA-ARS National Sedimentation Laboratory / Water Quality and Ecology Research Unit, USDA-ARS National Sedimentation Lab / Water Quality and Ecological Processes Unit; M.A. Locke, USDA-ARS National

Sedimentation Laboratory / Water Quality and Ecology Research Unit; R.L. Bingner, USDA-ARS National Sedimentation Laboratory / Watershed Physical Processes Research Unit; S.S. Knight, R.W. Steinriede, USDA-ARS National Sedimentation Laboratory / Water Quality and Ecology Research Unit. Beasley Lake watershed in the Mississippi Delta is a 915 ha intensively cultivated watershed (49-78% in row crop production) that was monitored for the herbicide metolachlor from 1998-2009. As part of the USDA Conservation Effects Assessment Program (CEAP), the watershed was assessed for the effectiveness of agricultural best management practices (BMPs) implemented during the study period including vegetated buffer strips, conservation tillage, conservation reserve program (CRP), constructed wetlands, and quail habitat buffers. A variety of crops have been grown in the watershed including conventional tillage cotton, corn, milo, and conservation tillage soybeans. The purpose of the present study was to examine the influence of varying BMPs and/or land-use (cropping patterns) on observed metolachlor concentrations in Beasley Lake water. Metolachlor was applied to cotton, corn, milo, and soybeans for weed control during spring (March-May) of 1998-2002, 2005, and 2007-2008. During those years, average mass of metolachlor applied was 12.7 kg with peak application during 1998 and 2007-2008. Spring metolachlor concentrations were measured in Beasley Lake water approximately monthly during the study period. Spring metolachlor concentrations in Beasley Lake ranged from below detection (< 0.02 ppb) to 3.1 ppb with an average of 0.16 ppb. A simplified model was produced to predict lake water metolachlor concentrations based upon herbicide applications. The model was in good agreement ( $R^2 = 0.875$ ) when BMPs encompassed < 48% of the total watershed but in poor agreement ( $R^2 = 0.197$ ) when BMPs encompassed >53%. Linear regressions used to determine associations of metolachlor with BMPs and/or cropping patterns showed decreasing ratio of lake metolachlor mass:applied metolachlor mass with increasing conservation tillage ( $R^2 = 0.342$ ), CRP ( $R^2 = 0.309$ ), and all BMPs combined ( $R^2 = 0.458$ ) while ratio of lake metolachlor mass:applied metolachlor mass increased with increasing conventional tillage cotton ( $R^2 = 0.459$ ). As a result, metolachlor levels in Beasley Lake after herbicide application are mitigated >3.7-fold when >50% of the watershed have implemented BMPs and >3-fold when < 9% of the watershed area are planted in conventional tillage cotton. Thus, greater conservation tillage, CRP, and combined BMPs in conjunction with decreased conventional tillage cotton presumably leads to decreases in applied metolachlor reaching the lake.

**TP143 Big Wind and Solar Projects: Environmental Assessments and Cumulative Impacts in a Changing Regulatory Landscape** R.H. Podolsky, Exponent / Ecological Sciences. With the proliferation of both grid-scale wind and solar power projects, there has been a concomitant increase in the concern regarding both local and regional environmental impacts from such projects. This is reflected in the recently released version of the voluntary guidelines published by the U.S. Fish and Wildlife Service that go beyond their previous guidelines in terms of both pre- and post-construction monitoring and assessments. For wind power, this is due to the recognition that turbine collision mortality may be approaching 500,000 per year in the US and thereby raising concern regarding cumulative impacts. Similarly, grid scale solar is increasingly seen as co-opting many of the ecological services provided by ecosystems where it is installed. Here too, cumulative impacts to plant, birds, mammals and reptiles is an increasing concern of state, local and federal agencies. Scientific data pertaining to the loss of ecological services and to cumulative impacts from both grid-scaled wind and solar projects will be presented and put into the context of the regulatory framework that guides major renewable energy project development.

**TP144 Bioaccumulation Risk Assessment Modeling System (BRAMS): Software for Human Health & Ecological Risks in Dredged Sediment** K. Baker, U.S. Corps of Engineers / Engineer Research and Development Center; J. Vogel, U. S. Army Corps of Engineers / Engineer Research and Development Center; A. Tkackuk, U.S. Army Corps of Engineers; O. Pabst-Guza, Environmental Protection Agency / Region I; N. Farris, U. S. Army Corps of Engineers / New England District; I. Linkov, US Army Corps of Engineers / Engineer Research and Development Center, US Army Engineer Research and Development Center. Each year, several hundred million cubic yards of sediment are dredged from rivers, canals and harbors of the U.S. and transported to disposal sites around the country. This dredged material must be carefully evaluated to determine the risks posed by contaminants that may be harmful to human and ecological receptors at the proposed disposal site. In order to make timely and cost effective dredged

material management decisions while protecting human and ecological health, risk assessors must determine risks associated with bioaccumulation of contaminants in dredged sediment quickly and accurately. This means adapting existing models to specific areas and risk scenarios and updating relevant parameters. The Bioaccumulation Risk Assessment Modeling System (BRAMS) is a software tool that allows users to develop and adapt bioaccumulation models within defined frameworks to include current and site specific data to evaluate human health and ecological risks. The program utilizes a steady-state uptake model based on the approach of Gobas to estimate fish body burdens for hydrophobic organic compounds, trophic transfer factors from invertebrates to fish for certain metals, and bioconcentration factors from water to fish for the remaining metals and hydrophilic organic compounds. Additionally, one of the included models follows the USEPA screening level risk assessment framework. BRAMS users can create and edit risk receptors, contaminants, and environments and dictate food chain dynamics and trophic transfer calculation methods to tailor the risk assessment to specific sites and to compare results using different input parameters and calculation methods. Model outputs include total carcinogenic and non-carcinogenic risks to humans and risks to ecological receptors as well as risks from specific chemicals and dietary species. BRAMS is also capable of accounting for parameter uncertainty using trapezoidal fuzzy numbering.

**TP145 Comparison of Observed and Estimated Concentrations of Chlorinated PAHs Using a Gaussian Dispersion Model in the Vicinity of Waste Incinerator** Y. Miyake, University of Shizuoka / Institute for Environmental Sciences; Q. Wang, L. Tang, University of Shizuoka; Y. Horii, K. Nojiri, N. Ohtsuka, Center for Environmental Science in Saitama; T. Amagai, University of Shizuoka. Chlorinated polycyclic aromatic hydrocarbons (CIPAHs) such as chlorobenz[a]anthracene (ClBaA) and chlorobenzo[a]pyrene (ClBaP) have received worldwide attention because of their environmental persistence and widespread distribution. Horii *et al.* have showed that several CIPAHs and BrPAHs elicit dioxin-like activity with potencies comparable to those of several mono-ortho polychlorinated biphenyl (PCB) congeners. Recent reports have showed the occurrence of CIPAHs and brominated polycyclic aromatic hydrocarbons (BrPAHs) in flue gas and fly ash from municipal and industrial waste incinerators. In this study, we measured individual concentrations and emission rates of 26 CIPAHs in stack gas from waste incinerators, and estimated the exposure concentrations of CIPAHs in the vicinity of incinerators by using a Gaussian dispersion model (Ministry of Economy, Trade and Industry—low-rise industrial source dispersion model: METI-LIS model) and the emission rates obtained in this study. Twenty six individual CIPAHs (mono to tetrachloroPAHs) determined in this study were monochlorofluorene (ClFle), mono to trichlorophenanthrene (Cl<sub>n</sub>Phe: n=1-3), mono to tetrachloroanthracene (Cl<sub>n</sub>Ant: n=1-4), mono to dichlorofluoranthene (Cl<sub>n</sub>Flu: n=1-2), mono to tetrachloropyrene (Cl<sub>n</sub>Pyr: n=1-4), mono to dichlorochrysene (Cl<sub>n</sub>Chr: n=1-2), mono to dichloro benz[a]anthracene (Cl<sub>n</sub>BaA: n= 1-2), and monochlorobenzo[a]pyrene.

**TP146 Demographic, Lifestyle and Dietary Correlates to Serum Dioxin Concentrations in Hot Spots at Vietnam Related to Continued Exposure to Agent Orange** D.T. Pham, University of Wyoming / Department of Zoology and Physiology (3166); H. Nguyen, Vietnamese Ministry of Natural Resources and Environment / Office of National Steering Committee 33; T. Boivin, Hatfield Consultants; A. Zajacova, University of Wyoming / Department of Sociology; H.L. Bergman, University of Wyoming / Department of Zoology & Physiology. Agent Orange was the main defoliant used by the US military in Vietnam from 1961-1971; this herbicide was contaminated with dioxin (2,3,7,8-tetrachlorodibenzo-*p*-dioxin, or TCDD). There are several identified major dioxin “hot spots” resulting from its use, distribution and spillage at former US military bases in Vietnam. Recent studies at 2 of these hot spots, Da Nang and Bien Hoa, have found high TCDD concentrations in all media exceeding international standards. Elevated dioxin concentrations in the food chain are likely sources of human dietary exposure, posing a very serious health risk for the Vietnamese population living on or near these hot spots. Our objective for this study is to evaluate potential demographic, lifestyle and dietary risk factors contributing to serum TCDD levels in Vietnamese residents at and near these contaminated sites. We used previously reported serum TCDD concentrations and extensive socioeconomic, lifestyle and food survey results related to potential dioxin exposure (Hatfield Consultants and Office 33 2009;



2011). We then analyzed these data using linear regression models; results from these analyses were then compared with those measured at other dioxin contaminated sites such as Seveso, Italy, Chapaeusk, Russia and Midland, Michigan. Preliminary results indicated potential water-related activities at the hot spots such as fishing and eating food collected at lakes and ponds were both related to blood TCDD levels. These results suggested new recommendations to decrease TCDD exposure for local residents living on or near these hot spots.

**TP147 Ecological Risk Assessment for Phase 3 of the TVA Kingston Ash Recovery Project; Roane County, TN** D. Jones, M. Beauchemin, ARCADIS; N. Bonnevie, ARCADIS / Risk Assessment and Ecological Services; D. Buys, ARCADIS; L. Fontenot, B. Fulton, ARCADIS / Risk Assessment and Ecological Sciences; C. Meyer, ARCADIS; J. Meyer, Arcadis U.S., Inc., ARCADIS; D. Rigg, T. Schlegel, A.R. Stojak, M. Wacksman, ARCADIS; S. Young, ARCADIS, ARCADIS / Environmental; N. Carriker, Tennessee Valley Authority. The December 2008 Kingston ash release discharged approximately 5.4 million cubic yards of coal ash slurry into the adjoining river system. The initial ash release at Emory River mile (ERM) 2.5 traveled upriver as far as ERM 5.75, with some ash also being transported downstream into the Clinch River and Tennessee River. In response to this event, the TVA Kingston Ash Recovery Project was executed in three distinct phases. Phases 1 and 2 focused on mass removal of ash from the Emory River and impacted embayments, as well as closure of the failed dredge cell. Phase 3 included comprehensive human health and ecological risk assessments of the estimated 500,000 CYs of residual ash that were not removed during Phase 1 or were transported downstream during storm events. The Baseline Ecological Risk Assessment (BERA) evaluated a wide-ranging list of potentially exposed and sensitive ecological receptors. The 17 assessment endpoints included maintenance and reproduction of balanced communities or populations of fishes, benthic invertebrates, aquatic plants, birds, mammals, amphibians, and reptiles. An extensive suite of biological and environmental data was collected for this assessment, resulting in multiple lines of evidence (LOEs) for most receptors. A weight of evidence (WOE) paradigm was used to evaluate multiple LOEs in the risk characterization process. Potential risk was categorized as Negligible, Low, Moderate, or High. Confidence in the risk determination was categorized as being Low, Moderate, or High. The final characterization of risk also included a determination as to whether risk management actions were recommended for an assessment endpoint. This recommendation was based on the WOE and provided a means for further characterizing the likelihood and magnitude of impairment of the assessment endpoint. Overall, the estimated risks related to residual ash and ash-related constituents were estimated to be: 1) primarily low and, at most, moderate; 2) associated with direct exposures of benthic invertebrates to ash, arsenic, and selenium in surface sediment, and dietary exposures of birds to arsenic and selenium via consumption of benthic invertebrates; and 3) localized and diminished with increasing distance from the release area. This information was considered along with other factors in the EE/CA in order to support the risk management decision-making process for the river system.

**TP148 Ecological Risk Management for Phase 3 of the TVA Kingston Ash Recovery Project; Roane County, TN** C. Zeller, US EPA – Region 4 / Environmental Engineer. On May 11, 2009, the Tennessee Valley Authority (TVA) entered into an Administrative Order on Consent (AOC) with the Region 4 Office of the U.S. Environmental Protection Agency (EPA), under the regulatory authority of the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA), to address the approximate 5.4 million cubic yards (CYs) of coal ash released to the environment on December 22, 2008. The TVA Kingston Ash Recovery Project was divided into 3 distinct phases using Time Critical and Non Time Critical Removal Action authority under CERCLA. Phase 1 was a Time Critical Removal Action to mitigate potential upstream flooding and downstream transport of ash. Phase 1 consisted of hydraulic dredging, mechanical excavation, ash dewatering, rapid materials handling, and off-site rail transportation and disposal of 3.5 million CYs of ash. Approximately 4.0 million tons of ash recovered from the river was safely transported via 414 trains to the approved Arrowhead Landfill in Perry County, Alabama. Phase 1 activities were completed in December 2010. Phase 2 is a Non-Time Critical Removal Action that involves mechanical excavation of approximately 2.8 million CYs of ash in the north and middle Swan Pond Embayments of Watts Bar Reservoir. Recovered ash is consolidated on-site in a 250 acre disposal area

that is being re-engineered with a subsurface perimeter stabilization wall to withstand liquefaction forces caused by the design seismic events. When ash consolidation and wall construction is completed, the cell will be capped and covered. Phase 2 construction will be completed in 2014. Phase 3 involves a comprehensive human health and ecological risk assessment of the estimated 500,000 CYs of residual ash that was not removed during Phase 1 or was transported downstream during storm events. The Phase 3 Baseline Ecological Risk Assessment (BERA) evaluated 17 measurement endpoints for coal ash related impacts. In addition, extensive geochemistry studies, sediment/pore water bioassays, benthic macro invertebrate assessments, 2 dimensional sediment-ash fate/transport modeling, and groundwater modeling (MODFLOW) were conducted. This platform presentation will discuss the removal alternatives evaluated in the Phase 3 Engineering Evaluation/ Cost Analysis Report, and how ecological risk management was factored into the selection of the 3<sup>rd</sup> and Final Action Memorandum for the project.

**TP149 Ecosystem Services as a new paradigm for Environmental and Social Impact Assessment—Implications for large development projects** P. Booth, Exponent / EcoSciences; S. Law, Exponent / EcoSciences, Exponent / Environmental Group. There is tremendous momentum in the public planning sector for using the concept of Ecosystem Services (ES) as a means of implementing holistic resource management. During the past 4-5 years, this momentum has spilled over into the private sector, in part driven by multilateral banks such as the International Finance Corporation of the World Bank (IFC) and the InterAmerican Development Bank (IDB). In particular, IFC recently adopted significant revisions to Performance Standard 6 with objectives that include protection and conservation of biodiversity and maintenance of the benefits from ecosystem services. IFC's updated Performance Standard 6 along with its detailed Guidance Note is resulting in the need for project developers to revise the way ESAs are performed if they are to seek financing from the over 77 commercial banks that have adopted IFC guidance as part of lending policy and requirements. Several NGOs are also actively engaging with lenders as well as the private sector in developing guidance that will affect the way ESAs are conducted. The road ahead is unclear not just due to evolving guidance, but also a lack of technical clarity or precedent. This presentation will provide an overview of ES in ESIA, including the driving forces, key technical and strategic considerations, and recommendations for Project developers.

**TP150 Enhanced Adaptive Management: Application to the Everglades Ecosystem** C. Foran, US Army Corps of Engineers / Engineer Research and Development Center, Army Engineer Research and Development Center / Environmental Laboratory; M. Convertino, University of Florida; A. LoSchiavo, U. S. Corps of Engineers / Jacksonville District; I. Linkov, US Army Corps of Engineers / Engineer Research and Development Center, US Army Engineer Research and Development Center. Adaptive management (AM) has been increasingly recognized as the best management strategy to move forward in the face of uncertainty and generate new learning to improve actions that increase chances for success. However, many applications have been criticized for shortcomings. Specifically, AM should provide scientific feedback on the robustness and flexibility of the proposed alternative, promote learning and prioritize monitoring needs. While several approaches have been utilized, many of these fail to realize the potential of AM. Decision-analysis models are needed to provide managers and scientists with transparent and easy to use tools to evaluate management alternatives and plans, the uncertainty and risk associated with each option, and the values associated with achieving multiple, often, competing objectives. We have developed a decision tool which is designed to guide initial selection of management alternatives, but also allows for adjustment based on monitoring information. This presentation will demonstrate the use of this tool and the way in which its outcomes can inform the choice of management alternatives for decomposition of water conservation areas in Florida. The process demonstrates that having a decision analysis tool can help in linking science, monitoring information, and management choices to select or adjust management alternatives in order to achieve program/project goals and objectives.

**TP151 Environmental and Social Risk Management Tools for Mining Projects – Red Dog Alaska Case Study** S. Shock, Exponent; R. Hager, Teck Alaska Incorporated. Public concern can be a major factor impacting the viability and cost of major development projects such as with mine sites. In some cases, it can either block or shut down a project. In order to better

manage these risk factors, a comprehensive risk management approach is needed. This presentation will describe a risk management methodology—developed at Red Dog Mine in Alaska—that is broadly applicable during development of new mine projects, expansion of existing projects, or during and following closure of existing mines. At Red Dog Mine, stakeholder workshops were used to identify and develop a set of clearly stated risk management objectives to address metals-bearing fugitive dust. These objectives were used as the basis upon which to develop a Risk Management Plan and program designed to achieve these objectives. Within the framework of the Risk Management Plan, implementation plans were developed to address specific risk management objectives. These implementation plans included a Communication Plan, Dust Emissions Reduction Plan, Worker Dust Protection Plan, Monitoring Plan, Remediation Plan, and an Uncertainty Reduction Plan. The plans incorporate periodic review to so that ongoing improvements can be incorporated to improve effectiveness at meeting the underlying risk management objectives. At Red Dog Mine, this risk management program is integrated with the Red Dog Environmental Management Systems database to ensure documentation and tracking of task assignments, roles and responsibilities, and to ensure timely completion of risk management tasks. This program has been effective at engaging stakeholders, including Alaskan native communities that have subsistence use activities in the vicinity of the mine, in a partnership to manage and minimize risks from fugitive metals dust to human health and the ecological receptors in the tundra environment over the life of the mine and into post-closure management.

**TP152 GLP/Guideline and Non-GLP/Research Studies Can Co-Exist in Regulatory Decision-Making Paradigms** W. Hillwalker, Oregon State University / Department of Environmental & Molecular Toxicology, Exponent / Chemical Regulation and Food Safety Center; J. Staveley, Exponent / EcoSciences. In the era of REACH, of new and emerging health concerns and of improved detection of environmental contaminants, risk assessment is of utmost importance in regulatory decision making. Regulatory frameworks developed initially in North America and Europe, and which are being increasingly adopted by other geographies, rely on the use of standardized testing guidelines and Good Laboratory Practices (GLP) to generate reliable and valid data to protect human health and the environment. In recent years, these same regulatory frameworks have emphasized economy in use of resources for testing and mandate the use of existing data whenever possible, for example non-GLP studies published in peer-reviewed journals, on a weight of evidence (WoE) approach. However, there is a growing disenchantment among researchers that regulatory agencies are giving special prominence to industry-funded GLP studies over non-GLP studies conducted with government funding. The concerns raised about GLP studies are mis-identified; the argument should really be about the suitability of the study design, transparency and access to raw data, and development of criteria for evaluation of all information that may be used in a WoE assessment. Focusing on the dichotomy between industry-funded GLP studies versus government-funded non-GLP studies muddies the issue. The global acceptance of GLP and testing guidelines and the significance and challenges of both standardized testing guidelines and non-standardized, non-GLP studies for the generation of critical data for risk assessments is discussed. Guidance on steps that can help to improve the reliability and validity of all data used in regulatory risk assessments is provided.

**TP153 Incorporation of relative bioavailability into risk assessment and remedial decision: A case study in the As-contaminated former smelter area, Korea** K. Yang, B. Jeong, Seoul National University / Dept. of Civil and Environmental Engineering; K. Nam, Seoul National University / Department of Civil and Environmental Engineering, Seoul National University / Dept. of Civil and Environmental Engineering. Consideration of site-specific bioavailability of heavy metals in soil is important in performing risk assessment and in determining relevant cleanup goal and an appropriate remediation technology. As an alternative to the bioavailability that requires *in-vivo* test, the relative bioavailability (RBA) based on bioaccessibility (results of *in-vitro* test) can be employed. In this study, the bioaccessibility and the chemical forms of arsenic present in a former smelter site in Korea were assessed. Total arsenic concentrations determined by *aqua regia* ranged from 21.3 to 120 mg/kg (avg. of  $75.4 \pm 35.1$  mg/kg) and nine out of ten surface soil samples exceeded the worrisome level of the Korean Soil Regulatory Level for arsenic (25 mg/kg). The bioaccessibility of arsenic was further determined with the method proposed by the Solubility/Bioavailability

Research Consortium (SBRC) method (Kelly et al., 2002, Assessing Oral Bioavailability of Metals in Soil). The bioaccessible arsenic fraction ranged from 2.1 to 15 mg/kg (avg.  $7.0 \pm 4.4$  mg/kg), resulting in 3.0 to 26% (avg.  $12 \pm 8.0\%$ ) of bioaccessibility (i.e., the ratio of the bioaccessible fraction to the total concentration). A sequential extraction procedure developed by Wenzel et al. (Analytica Chimica Acta, 2001, 436, 309-323) was adopted to analyze the chemical forms of arsenic in soil samples. The majority of arsenic was present as the residual fraction (i.e., 60–98%, avg.  $80 \pm 14\%$ ), and the remainders were as  $\text{SO}_4$  extractable fraction,  $\text{PO}_4$  extractable fraction, and amorphous and crystalline Fe/Al oxyhydroxide fractions. Our results show that site-specific RBA is essential in determining realistic risk of the arsenic-contaminated site. Through the extensive site survey to determine RBA, the cleanup goal of arsenic will be determined and the remedial strategy for the former smelter site will be suggested.

**TP154 Managing Risk Assessment and Risk Communication Differences Between Responder and Community in Chemical Emergency Response Scenarios** A. Pawlisz, Conestoga-Rovers & Associates. Increased global threats and calls for improved national security associated with the production, transportation, and handling of hazardous materials have behooved emergency responders to heighten their awareness of the need for appropriate risk assessment tools. Typically, emergency-scale chemical releases have sudden and catastrophic impacts and rapid response/risk assessment is of essence when controlling/minimizing damage. One of tools used by responders are the occupational exposure limits that have been developed by various agencies with worker protection in mind. In addition to being unequivocal in terms of values, exposure scenarios, and the level of protection, they may also not be entirely applicable to gauge community health issues in an area affected by the spill. This presentation discusses the range of occupational limits available, their intrachemical variability, and applicability to various emergency response scenarios. The presentation also elaborates on the options for the community health risk assessment benchmarks and how they differ from those for emergency worker exposure. Suggestions are offered on how to effectively communicate the dissimilar goals to the multidisciplinary responder team and the public.

**TP155 Methylene Chloride – Common Lab Contaminant or Mutagen?** E. Krupka, CDM Smith. Methylene chloride is an industrial chemical which has been used historically in paint strippers, as a propellant in aerosols, as a metal cleaning solvent and in the manufacture of drugs, pharmaceuticals, film coatings, electronics, and polyurethane foam, and during the decaffeination process of coffee and tea. Production of methylene chloride has declined since 1980, but it continues to maintain its prevalence in the environment. Additionally, methylene chloride is used in conducting laboratory extractions in the analysis of environmental samples. Methylene chloride is often detected in environmental samples at low concentrations and is considered to be a common laboratory contaminant. In November of 2011, the U.S. Environmental Protection Agency (EPA) concluded that methylene chloride is carcinogenic via a mutagenic mode of action. In animal studies, methylene chloride has been found to induce DNA-damage and tumor formation in target tissues in the liver and lung. Glutathione S-transferase-theta1-1 (GST-T1) has been found to catalyze a metabolic pathway which produces DNA-reactive metabolites responsible for this DNA damage in mice. The GST-T1 metabolic pathway has also been identified in human tissues; therefore increased early-life susceptibility is assumed in humans. EPA has also recommended the use of age-dependent adjustment factors when estimating age-specific cancer risks to account for early-life sensitivity. This, in conjunction with recently released decreased toxicity values for methylene chloride, has the potential to lead to increased risk from exposure to relatively low levels of this contaminant. In light of this information, it is imperative to reassess current attitudes regarding methylene chloride use and exposure.

**TP156 NATO Sustainability Symposium Review** I. Linkov, US Army Corps of Engineers / Engineer Research and Development Center, US Army Engineer Research and Development Center. Risk analysis and sustainable planning go hand-in-hand, and are especially important for small settlements and military installations. Access to reliable and affordable energy and water resources is important to ensure a high quality of life in civilian communities and mission success at military installations. Key to successful sustainability planning is accounting for natural and man-made risks, such as impacts of climate change, terrorist attacks, and other

disruptions that may radically impact the efficacy of sustainability strategies and technologies. Even though significant resources have been directed to predict potential consequences of climate change and other future scenarios, additional emphasis is needed to develop rational approaches in order to guide decision-making under uncertainty and methods to develop and compare the performance of alternative adaptive strategies within an overall adaptive management approach. There is a need for novel methods to aid planners in considering risks and make tradeoffs when designing a resilient sustainability strategy. The objective of this symposium is to summarize the insights gained at the "Sustainable Cities and Military Installations" workshop (3-6 June 2012 in Hella, Iceland) hosted by NATO and supported by SRA. The NATO workshop will review the state-of-the-science in current and emerging technologies, methods, and frameworks for energy and water conservation, efficiency, and renewable energy within the context of climate change, with an emphasis on environmental impacts, security, and military-readiness and effectiveness.

**TP157 Natural Mitigation of Acidic Mine Soil: Evaluation of the Atmospheric Deposition of Alkaline Minerals** C. Meyer, J. Gillow, ARCADIS;

K. Pratt, University of California San Diego; R. Lindeman, A. Thatcher, M. Barkley, J. Sueker, P. Hunter, ARCADIS; K. Prather, University of California San Diego. Unusual atmospheric deposition events should be considered when evaluating remedial alternatives. In this case study, sulfuric acid deposition that resulted from the smelting of sulfidic ore to refine copper has acidified soil at a mine site. The acidic soil enhances the mobility of metals in soil, including copper. Soil neutralization, typically achieved through engineered solutions such as liming, can decrease metal mobility. In 2008, a natural rainfall event that contained dust from salt playas up to 100 miles from the mine deposited calcium-rich minerals that increased the pH of the soils at the mine site, essentially liming the soils naturally. The chemistry of the rainfall was sampled from various locations to analyze the major and trace elements of dissolved and suspended solids. The "white" rainwater was characterized by playa dust residues containing calcium oxide and calcium hydroxide, in addition to aluminosilicates. Sampling after the "white" rain event verified a significant increase in soil pH (>1.3 units) in upland locations having pH values ranging from 3.7 to 5.4. The pH did not significantly change in soil locations with higher pH values. The pH change was monitored annually on the mine site through 2011. During that time, soil pH values as a result of the "white" rain were maintained or increased in most low-pH (< 5) locations and were maintained or decreased in most high-pH (>6) locations, although additional years of monitoring are needed to confirm these trends. A high pH (>6.5) in soils was maintained or increased in a few areas that contained carbonate parent rock. Speciation of the minerals in the soil indicates that the soils contain some potential acidity in the form of copper sulfide minerals (e.g., cubanite, chalcopyrite, bornite, covellite) that could cause the pH to revert back to lower levels. However, a comparison of acid base accounting results with sulfide mineral percentages indicates that a relative minority of the soils are strongly acid generating. The relatively low acid generation potential suggests that the natural "white" rain event may have permanently increased soil pH in many of the locations. The high pH (>6.5), combined with high calcium (>5.1 mg/L) observed in the "white" rain, is unusual, recorded only twice before in the last 26 years (19 and 21 years prior to this event). Such unusual atmospheric deposition events make natural attenuation a viable remedial alternative to consider.

**TP158 Prioritizing marine spatial planning efforts with the assets, threats and solvability framework** J.F. Carriger, Jr., U.S. EPA, U.S. EPA / Gulf Ecology Division; S.H. Yee, U.S. EPA, U.S. EPA / Gulf Ecology Division.

The application of marine zoning and spatial planning methods has seen a worldwide increase to stem unsustainable use of coastal seas. However, prioritizing marine regions for focused management efforts and protection can be a difficult process. Uncertainties from the provision of benefits, the threats to those benefits, and future success of management can be great in a marine region. The assets, threats, and solvability (ATS) model was adapted and modified for spatially explicit prioritization of marine planning. The ATS framework offers capabilities for screening level assessments of marine regions in order to target high priority areas for management which can be followed with more detailed field monitoring and valuation. The ATS framework is being applied in developing management prioritization maps for coastal seas adjacent to Puerto Rico and the U.S. Virgin Islands. Spatial layers describing benthic habitat types, stressor location and intensity, and boundaries for marine protected areas were collected and related functionally

through probabilistic Bayesian networks. Coupling the Bayesian networks to the ATS framework provides better characterization of uncertainties in spatial relationships, and consequently uncertainties in prioritization of different areas. The Bayesian network may further be used to infer how alternate management scenarios influence the distribution and intensity of assets and threats. Results from this work will allow us to evaluate the potential of the framework for supporting management objectives and the spatial planning process.

**TP159 Relationship between physicochemical parameters and algal community at Kamfers Dam, a saline lake in Kimberley, South Africa** L. Moore, Clemson University / School of Agriculture, Forest and Environmental Sciences; W. Bowerman, University of Maryland / Department of Environmental Science and Technology; W. Bridges, Clemson University / Department of Mathematical Sciences.

Kamfers Dam, a large wetland situated in Kimberley, South Africa, was home to over 80,000 breeding lesser flamingos (*Phoeniconaias minor*), a near-threatened species. *Arthrospira fusiformis*, the flamingos preferred food source, accounted for 99.5% of the algal cells present and was found at extremely high concentrations, over six million cells per milliliter, as recently as April 2009. The city's waste water treatment outflow drains directly into this water body and is now operating over maximum capacity and discharging untreated sewage water directly into Kamfers Dam. This is creating elevated water levels, extremely poor water quality and hyper-eutrophication. A crash in the *Arthrospira* population has compromised the food source of the lesser flamingo population and allowed other algal species to bloom and become dominant including toxin producing species. As a portion of an ecological risk assessment, the water quality and algal community of Kamfers Dam were monitored using historical water quality data, as well as new water quality analyses, in April 2009, March 2010, September 2010, and August 2011. Several parameters showed a statistically significant decrease over time (ANOVA,  $p < 0.05$ ) including pH, conductivity, sodium, chloride, and total dissolved salts. Spearman rank correlation was used to measure relationships among physicochemical parameters and the densities of algal classes and/or species. Cyanophyceae was negatively correlated with Bacillariophyceae (Spearman  $\rho = -0.541$ ,  $p = 0.046$ ) and Chlorophyceae (Spearman  $\rho = -0.825$ ,  $p = 0.0003$ ). *A. fusiformis* was positively correlated with conductivity (Spearman  $\rho = 0.561$ ,  $p = 0.029$ ), total dissolved salts (Spearman  $\rho = 0.572$ ,  $p = 0.026$ ) and negatively correlated with total phosphorous (Spearman  $\rho = -0.718$ ,  $p = 0.003$ ). While significant correlations were found, attempts to develop a model for predicting algal community composition, using several of the parameters and algal group densities in combination, was unsuccessful due to strong multicollinearity among the parameters and algal group densities.

**TP160 Responding to Community Concerns Surrounding Hydraulic Fracturing Activities near a School** K.M. Hitchcock, ChemRisk / Associate Health Scientist; J. Panko, P. Scott, E. Shay, K. Unice, ChemRisk.

The number of active hydraulic fracturing sites continues to grow in Western Pennsylvania, and along with them, community concerns. Often monitoring data are not always available to answer the questions that community members have. When a new well was drilled approximately 900 yards from the Fort Cherry school campus (McDonald, Pennsylvania), local parents expressed concerns over the students' potential for exposure to chemicals related to hydraulic fracturing. To answer their concerns, we developed an air monitoring program. Meteorological conditions (wind direction, wind speed, temperature, humidity, precipitation, and barometric pressure) were measured using a weather station. Continuous air monitoring to sample for volatile organic compounds (VOCs), hydrogen sulfide ( $H_2S$ ), and explosive gases [measured in terms of the percent of the lower explosive limit (LEL)] was conducted using a MultiRae Plus gas monitor, a ppbRAE 3000 VOC monitor, and a RAELink3 system. In addition, summa canisters were deployed to sample for individual VOCs. Data were collected at baseline (between November 14 and 29, 2011), during fracking activities (November 11 through December 27, 2011), and during flaring activities (December 28, 2011 through January 6, 2012). The wind was predominantly from the southwest during all sampling periods, placing the school upwind of the well pad. All explosive gas measurements were 0% LEL during each sampling period. Daily average  $H_2S$  ranged from non-detect (ND) to 0.14 ppm (baseline), 0.00035 to 0.15 ppm (fracking), ND to 0.15 ppm (flaring). Daily average total VOCs ranged from ND to 0.48 ppm (as measured by the MultiRae) and ND to 0.385 ppm (as measured by the ppbRae) during baseline; from ND to 0.36 ppm (MultiRae) and ND to 0.031 ppm (ppbRae)



during fracking; and from 0.055 to 0.52 (MultiRae) and ND to 0.022 ppm (ppbRae) during flaring. In general, we found no remarkable results as compared to background levels, and a basic screening level assessment showed that the measured VOCs were below health based screening levels. All results were communicated to the school board. Our example shows how very basic risk assessment techniques can be used to fill inherent data gaps and address community concerns related to hydraulic fracturing.

**TP161 Risk Communication in the World of Social Media** A. Van Ryan, ARCADIS, Inc.. With apologies to Bob Dylan...the times they are certainly a' changing, especially when it comes to how trust is being assigned and information evaluated by the various audiences we need to involve in the communication of ecological and human health risk. Until recently, it was possible to explain the "complex and technical" through printed materials and public meetings alone. In the past five years, the arena of communications has evolved at an ever accelerating rate due to the slow demise of the print news media and nearly universal use of real-time electronic devices and social networking websites. Add to that the directive of USEPA's new Community Engagement Initiative to include those individuals or groups that may be "missed" by traditional outreach and involvement strategies, and risk managers are at the door of a new world of environmental communications. The methods of a risk communicator must also evolve in order to build the public's confidence and trust, maintain a consistent message, and manage the line between apathy and outrage. This presentation will use two case studies to demonstrate how successful risk communication is possible in this new world of social media. One example is the "Cheeseburger a Day" approach, in which risk communicators maintain a real-time flow of information using multiple channels, including print media, social media, and one-on-one personal meetings – even when there is nothing new to express. Traditional tools, such as simple yet strategic upfront attitude and awareness research, help guide the risk communication path forward by mining the viability of and best approaches for using electronic and social media.

**TP162 Scientific Management Decision Point within Ecological Risk Assessment: A case Study at US Department of Energy Hanford Site** C.J. McCarthy, CH2M Hill / Environmental Services; A. Aly, D. Morgans, Intera; J. Hansen, U.S. Department of Energy, Richland Operations Office; D. Wright, CH2M Hill. Ecological Risk Assessments are being completed as part of the Remedial Investigation/Feasibility Study process for CERCLA sites along the Columbia River and the Hanford Site. Within the ecological risk assessments, a final scientific management decision point (SMDP) occurs at which risk managers, risk assessors, and other stakeholders must agree on a path forward with respect to ecological risk associated with a site. Agreement must also be reached on the COCs, assessment endpoints, exposure pathways, and the risk questions associated with risk outcomes (e.g., no unacceptable risk, risk warranting remedial action, risk warranting further investigation). Factors that were considered to interpret the results of the risk characterization and determine if the site requires evaluation of remedial alternatives in the FS included the following: spatial characteristics; proximity and size of abutting un-impacted habitat; number, spatial distribution, and depth of analytical chemistry data; frequency that risk based thresholds are exceeded and the location(s) of those exceedances; properties of chemicals exceeding thresholds; basis and confidence of the threshold that is exceeded including which receptor and methods used to develop the threshold; consideration of home range and area use factors; and tying the risk back to the population and community level assessment endpoints. This information is presented within a matrix along with maps to aide in developing risk management recommendations. For completion of remedial actions as part of the cleanup verification process for future unaddressed sites, a SMDP process will be implemented on a case-by-case basis to determine that the action is protective of ecological receptors.

**TP163 Sediment Investigation of the An-Shun Remediation Site, Tainan City, Taiwan** B.J. Mastin, D. Renfrew, Weston Solutions Inc / Natural Resources; D.W. Moore, Weston Solutions, Inc / Director Natural Resource Management, Weston Solutions, Inc. / Water and Resource Management; J. Chen, Weston Solutions Taiwan Limited / Natural Resources. There are several challenges to performing environmental remediation and restoration projects in Taiwan including but not limited to a lack of established protocols for development of risk-driven clean-up guidelines. Taiwan is in the process of developing sediment and water quality criteria, health and safety regulations, and guidelines to establish site-specific remediation goals.

Without these decision-making guidance components, it is extremely difficult to obtain consensus among stakeholders on detailed scopes of work and subsequently negotiate achievable performance goals. Additionally, the Taiwanese Environmental Protection Bureau has not promoted the concept of performing preliminary Remedial Investigations and Feasibility Evaluations in order to develop a mitigation approach with the best potential for success to meet project goals. The overall objective of the An-Shun Remediation Project includes a multi-phased approach for the investigation, design engineering, and implementation of a full-scale project for the mitigation of mercury (Hg) and dioxin contaminated sediment in Seawater Pond B. These sediments were contaminated by the manufacturing of chlorine, sodium hydroxide, and pentachlorophenol over a greater than 40-year period at the site. The specific objective in this initial phase of the project was to perform a remedial investigation to determine the extent and magnitude of mercury and dioxin contaminated sediment in Pond B. Activities included conducting a comprehensive hydrograph and transect survey and collection of sediments cores for analyses of mercury, dioxins, particle size, percent solids, and total organic carbon (TOC). Sediment cores (49) were collected to a target depth of 3.0-m below the sediment water interface or until refusal was encountered. Sediments consisted of fine sands (61.6 to 77.8 percent) and medium sands (11.3 to 25.2 percent). Mercury and dioxin were analyzed in the 90-cm, 180-cm, and 300-cm horizons to delineate the horizontal and vertical extent of contamination and results ranged from ND to 862 mg/kg and 0.401 to 36,988 ng/kg dry weight, respectively. Percent solids and TOC ranged from 72.4 to 83.4 and 1.66 to 3.9 µg C/mg, respectively. These results will be used for defining the volume of sediment required for excavation and provide information necessary for the subsequent feasibility evaluation, full-scale sediment remediation design and engineering, and implementation of future project work.

**TP164 Sources of PAHs in Washington State and Recommendations for Human Health and the Environment** H. Davies, Washington Department of Ecology / Waste 2 Resources, St of Washington / Dept of Ecology; J.W. White, Washington Dept of Health / Health; A. Stone, Wa Department Of Ecology / Chemist; J. Grice, Washington Dept. of Ecology; K. Patora, Washington Dept. of Ecology / Economic Analysis Department; M. Kadlec, Washington Dept. of Ecology / Air Quality Program; D.A. Delistraty, Washington State Department of Ecology / Hazardous Waste and Toxics Reduction Program; D. Norton, Washington State Dept of Ecology / Environmental Assessment Program. The polycyclic aromatic hydrocarbon (PAH) chemical action plan (CAP) is part of the Washington State Department of Ecology's strategy to reduce threats from persistent, bioaccumulative, and toxic chemicals (PBTs). The goal of the PBT program is to reduce and phase-out PBT uses, releases, and exposures in Washington to protect human health and the environment. The PAH CAP was developed by the Departments of Ecology and Health with input from other state agencies, local government, businesses, and health and environmental organizations. To prepare the PAH CAP, data on adverse health effects of PAHs on humans and wildlife, their prevalence and concentration in environmental media, and their estimated releases from various sources in Washington State, were compiled from published scientific literature, state and federal government reports, and industry and environmental groups. The CAP includes recommendations to reduce PAHs, while considering existing laws and regulations, as well as economic impact. There are hundreds of PAHs and PAH derivatives. Adverse health effects are typically caused by exposure to complex mixtures of these compounds, rather than exposure to a single PAH compound. PAHs are primarily emitted by anthropogenic activities, although some natural sources also exist. PAHs are present in petroleum and are formed during incomplete combustion of organic matter such as coal, oil, gas, wood, garbage, and other materials, such as tobacco and meat. For most individuals, the largest exposures to PAHs are from food and smoking, with a lesser contribution from air emissions. For wildlife, the primary concern is sediment, serving as an environmental sink, where PAHs accumulate. For example, sediment PAHs can enter the aquatic food chain, accumulating in higher trophic levels, including wildlife and humans. We found that the largest anthropogenic sources to the environment in Washington State are wood burning stoves, creosote treated wood, and vehicle emissions, including tire wear, motor oil disposal and leaks. Because PAH exposure is linked to many sources, an effective program to reduce releases and exposure will require a multifaceted approach, including education, continued environmental testing, reduction of existing sources, and prevention of new sources. We present a series of initial actions that address

the worst sources of exposures, along with options for further actions in the future, as needed.

**TP165 Street Dust: Implications For Stormwater And Air Quality, and Management Through Street Sweeping** S. Calvillo, Baylor University / Center for Reservoir and Aquatic Systems Research, Department of Environmental Science; S. Williams, Baylor University, Baylor University; B.W. Brooks, Baylor University / Environmental Science and Biomedical Studies, Baylor University / Department of Environmental Science. Street dust represents a source of dual potential risk to stormwater and air quality. It has been well-documented that washing of this material to local watersheds can degrade water quality. Studies have also demonstrated that as much as 85% of ambient particulate matter (PM<sub>10</sub>), exposure to which is associated with several health effects, can arise from accumulated street dust. Furthermore, many contaminants, including metals, are at higher concentrations in the smallest available particles, which represent an important management issue for stormwater. Though street cleaning is considered a best management practice (BMP) by U.S. EPA, developing a comprehensive understanding of the efficacy of various street cleaning technologies and practices for stormwater quality was the purpose of the present study. The objectives of this study were to: (1) Develop an understanding of available street sweeping technologies and their relative efficacy. (2) Critically review the available literature regarding street dust and potential impacts on stormwater and air quality. (3) Extrapolate the relative efficacy of multiple street sweeping technologies to the context of environmental/ecological and human health risk. During this exercise, 1187 journal articles from the peer reviewed literature containing the phrases "street dust," "road dust," "urban dust," "roadway sediments," and similar search terms were reviewed. Two peer-reviewed studies compare the effectiveness of street sweeping technologies in the removal of street dust, though several government reports also exist. However, no attempts have been made to integrate these comparisons with the potential for risk to humans and the environment. Herein, we propose a framework to determine margins of safety for stormwater hazards and human health under multiple cleaning strategies and employing the available technologies. We specifically identify research needs to maximize street cleaning effectiveness as a stormwater management BMP.

**TP166 The Industrial Food Web as a Chemical Transport Network** C.A. Ng, ETH Zurich / Inst. for Chemical and Bioengineering, S&U Group, ETH Zurich / Institute for Chemical and Bioengineering; K. Hungerbuehler, Swiss Federal Institute of Technology (ETH Zurich) / Institute for Chemical and Bioengineering. The industrialization of the global food system means foods are increasingly produced in a limited number of regions, with food security implications not only for supply, but also for the movement of chemicals across borders and into human diets. Source-receptor models seek to determine which source regions account for chemicals in target regions, either by explicitly incorporating the flow of 'carrier media' (air, water) or by using mass balance models of tracer chemicals. Wind flow, water currents, diffusive processes and reactions in the atmosphere and in the oceans drive the transport of chemicals in these models. Human food web models, on the other hand, estimate the uptake of chemicals through the diet. These models largely rely on local food web descriptions. By limiting consumption to local sources, an integrated picture of chemical fate is possible, from emission to eventual uptake by humans. However, neither source-receptor nor human food web models explicitly incorporate the transport of foods across regions. Yet this picture is now the dominant reality for much of the world's population. A recent study by Hoekstra and Mekonnen (2012) to quantify the water footprint of humanity found that agricultural trade accounted for 92% of the 'virtual water flows' between countries. Thus, agricultural trade represents an important factor in the movement of material across regions. This movement is substantially different from that of 'natural' material carriers in the environment: while ocean and wind currents tend to slow transport across the equator, many major food flows are trans-equatorial (e.g. off-season fruits moving from the southern hemisphere to northern countries). Here, we show how substance flow analysis can be combined with chemical fate and bioaccumulation models to overlay the major distribution pathways of important foods with the environmental processes that drive chemical transport and bioaccumulation. We use this integrative approach to explore critical questions surrounding the local vs. global source-receptor relationships, regulatory landscape, and economic implications of the global industrial food system. With the approach outlined here, further light can be shed not only on how the global food

system affects human health risk but also on its status relative to principles of environmental justice.

**TP167 Treatment of Selenium in Produced Water Using a Pilot-scale Constructed Wetland Treatment System** M.M. Spacil, Diamond V / AquaSmart, Diamond V / Research & Technical Support; J. Rodgers, Clemson University / Forestry and Natural Resources, Clemson University / School of Agricultural, Forest and Environmental Sciences; J. CASTLE, Clemson University / Environmental Engineering & Earth Sciences; W.Y. Chao, Clemson University / Forestry & Natural Resources. Produced waters (PWs) containing potentially toxic concentrations of selenium (Se) may be generated as a byproduct of oil and gas recovery and can present a liability management challenge. Many of these PWs are fresh and could prove to be a great asset once treated for specific constituents (e.g. Se). Pilot-scale constructed wetland treatment systems (CWTs) were designed and built to evaluate Se removal from simulated fresh produced water (SFPW) aided by addition of an organic carbon and nutrient source to enhance dissimilatory Se reduction. Study objectives were to: (1) characterize a fresh PW and confirm Se as a constituent of concern (COC); (2) formulate a SFPW; (3) design and build a pilot-scale CWTs for SFPW; and (4) measure performance (i.e. COC removal rates and extents). The treatment goal for this study was to decrease the Se concentration in SFPW from >50 µg/L to ≤5 µg/L. To determine Se removal rates and extents and to evaluate environmental factors, measurements included elemental analysis of Se, dissolved oxygen, conductivity, pH, oxidation/reduction potential, alkalinity, hardness, and temperature. Mean outflow Se concentrations ranged from < 1 to 47 µg/L, depending on the treatment. Organic carbon amendments had a significant effect ( $\alpha = 0.05$ ) on Se removal demonstrated by increased removal rates and extents. This pilot-scale study illustrates that a suitable labile organic carbon source can improve Se removal in CWTs for PW and that stringent discharge limits can be achieved.

**TP168 Use of constructed wetlands to reduce pesticide concentrations in urban runoff** R. Budd, M. Ensminger, California Environmental Protection Agency / Department of Pesticide Regulation; K.S. Goh, California EPA / Department of Pesticide Regulation; S. Teh, University of California- Davis. Pesticides transported with urban runoff can negatively affect water quality of receiving waterways and often reach concentrations toxic to aquatic species. Constructed wetlands have proven an effective best management strategy in agricultural areas. This study will evaluate the efficiency of two small constructed wetlands receiving runoff from residential landscapes to reduce pesticide loading to receiving streams. Water and sediment samples will be collected at the inlets and outlet of each system, as well as water quality parameters recorded for each sampling event. The removal efficacy will be calculated for numerous insecticides including six pyrethroids, three organophosphates, fipronil and its degradates, imidacloprid, as well as four synthetic auxin herbicides. The list of analytes represents a wide range of physiochemical properties which influence transport behavior (sorption, degradation rates) within the systems. This allows for a more comprehensive evaluation of typical constituents in urban runoff than previously published studies. Water chemistry will be presented alongside toxicity data to evaluate improvement in ecosystem health. Vertical transport will be evaluated with samples collected from subsurface piezometers. In conjunction with measured flow data, the mass balance of chemicals within the systems will be presented.

**TP169 Water in Carbon Capture and Sequestration: Challenges and Opportunities** J. Rodgers, Clemson University / Forestry and Natural Resources, Clemson University / School of Agricultural, Forest and Environmental Sciences; J. CASTLE, Clemson University / Environmental Engineering & Earth Sciences; J. Wagner, Clemson University / Dept. of Environmental Engineering & Earth Sciences. Carbon capture and sequestration (CCS) technologies have the potential to greatly reduce anthropogenic carbon dioxide emissions to the atmosphere. The CCS process involves capture of CO<sub>2</sub> gas generated at large stationary sources such as thermoelectric power plants followed by compression and then transport to geologically favorable areas where the captured CO<sub>2</sub> is injected into subsurface units such as depleted oil and gas reservoirs, deep saline formations, or unminable coal seams. Because water consumption is estimated to increase by 50 to 90% with the addition of CO<sub>2</sub> capture and compression using existing technologies, new sources of water supply and augmentation of existing supplies will be needed. Although water production may not be

necessary during injection of CO<sub>2</sub> into geologic units for long-term sequestration, water production may be advantageous to increase storage volume and reduce pore pressure. Opportunities for beneficial use of sequestration produced waters (SPWs) will occur if energy-efficient and cost-effective treatment technologies are identified and implemented. Possibilities for beneficial use of SPWs include power-plant cooling and other industrial uses, irrigation, livestock watering, augmentation of surface water flow, and re-injection for pressure maintenance or waterflooding of oil and gas reservoirs. The potential for beneficial use of a specific SPW can be assessed by characterizing the SPW in terms of quantity and quality, including both chemical and ecological risks, and comparison with criteria or guidelines for various uses. Quantity and quality of SPWs vary with type (i.e. depleted oil and gas reservoirs, deep saline formations, or unminable coal seams) and are site-specific. SPWs contain a wide range and concentrations of cations, anions, and organic compounds. Treatment technology for a specific SPW should be selected based on water characteristics and intended use. Existing technologies with potential application to treating SPWs include reverse osmosis, thermal desalination, electrodialysis, freeze/thaw, and constructed wetland treatment systems. Preferred technologies will be those that provide effective treatment with a low energy demand. A range of treatment options is likely to be implemented for SPWs because of differences from site-to-site in water quantity, quality and potential uses.

**TP170 Deciding among Three Different Systems of Sewer Drainages to Preserve Ground Water in a Small Municipality in Buenos Aires Province** A. Gonzalez, National University of Lanus; L. Rius Blanco, National University of Lanus / Department of Productive Development And Technology; A.N. Monzon, National University of Lanus; S.O. Demichelis, Urban Environmental Management / Natural Sciences Faculty, National University of Lanus (UNLa) / Urban Environmental Management-Department of Productive Development and Technology. The aim of this study is to compare the environmental impact of three different sewer drainages: the traditional system of drainages, the condominium system, and the system free of solids, in order to evaluate the viability of implementation of each one in the municipality of Malvinas Argentinas, Buenos Aires Province. Located in the NW of Gran Buenos Aires (GBA), it is the district less favored as far as water infrastructure and sanitation services of this region with a 10.8% of potable water network and 2.1% of homes sewers. Most of the homes obtain drinkable water from water well and use cesspools to drain waters, some pollutants including fecal pollutants could be transported towards underground waters source of well. Considering these facts there are population in risk to parasitic and infectious diseases; therefore, the implementation of systems of water provision and basic sanitation are imperative. In this sense, the impact of three systems of houses wastewater drainages is analyzed in order to avoid the diffusion of fecal contamination towards underground waters. In the traditional system, the waste water is transported by domiciliary, collectors and maximum sewers, to finally be treated in plants. The required minimum depth for this system is of 0.80 m. The condominium system includes a branch, that collects from one block or condominium; it consists of a pipe, with smaller diameter than the traditional system, seated in protected zones, and the public networks that lead the water-drainages until the treatment system, both localized in a low depth. The system of sewage free of solids is based on the unloading of residual waters in an intercepting tank, where mud is digested anaerobically and must be retired each 5 to 10 years, being the residual liquid unloaded by gravity by means of pipes of small diameter and little depth, for its later treatment. Although the physical characteristics of the land in the area make possible the implementation of anyone of the three systems, and the use of the traditional system is accepted in Buenos Aires, the urgency that represents the water infrastructure deficit and sanitation services, added to the convenience costs of the alternative systems, makes the implementation of one of these last two more adequate. Nevertheless, we applied an impact study to decide between them. Its concretion will depend on the capacity of management and conciliation of the authorities with the community and other organisms.

**TP171 Preliminary Levels and Profiles of Persistent Organic Pollutants in Human Breast Milk from Colombia** B. Johnson-Restrepo, University of Cartagena / Chemistry in the Environment Group; E. Garcia-Cantillo, J. Olivero-Verbel, University of Cartagena. Concentrations of polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs), and organochlorine pesticides (OCPs) were measured in human breast milk samples. Samples (n=36) were collected in Cartagena city, Colombia from

mothers who have been feeding infants less than one year of old. Frozen milk samples were thawed out at 25 °C and 20 ml of the milk sample were extracted. Samples were spiked with <sup>13</sup>C-labeled surrogate standards. Liquid-liquid extraction was performed using 2.5 ml of 8% (w/w) potassium oxalate solution, 10 ml of ethanol, and 5 ml of diethyl ether were added to the samples. Samples were extracted twice with 5 ml of hexane. The extract was passed through a multilayer silica gel packed glass column for cleanup. The solvent was concentrated to 1 ml and was then injected into a gas chromatograph interfaced with a mass spectrometer (GC-MS; Agilent Technologies 7890 GC and 5975 Series MS). Concentrations of PBDE congeners found in the samples ranged from 0.03 to 810 ng/g lipid wt. BDE-47 (2,2',4,4'-tetraBDE), BDE-99 (2,2',4,4',5-pentaBDE), and BDE-100 (2,2',4,4',5-pentaBDE) were the major congeners detected in breast milk samples. Overall mean concentrations (mean ± SD) of PCBs, DDTs, HCHs, and HCB were 20.4 ± 2.62, 54.5 ± 5.5, 14.3 ± 9, and 12.6 ± 3.6 ng/g lipid wt, respectively. Concentrations of PBDEs were positively correlated with concentrations of PCBs and OCPs in the samples. Intake rates as daily ingestion rates of contaminants were calculated for infants. The estimated daily intakes of organohalogens were compared with the minimum risk level (MRL) suggested by the Agency for Toxic Substances and Disease Registry (ATSDR).

**TP172 Puente Alsina Slum** M.H Bracca, National University of Lanus / Department of Productive Development and Technology; C. Rojas Collingwood, A.N. Monzon, National University of Lanus; S.O. Demichelis, Urban Environmental Management / Natural Sciences Faculty, National University of Lanus (UNLa) / Urban Environmental Management-Department of Productive Development and Technology. The main objective of the present work is diminish vulnerability of this slum by the development of basic sanitation services like networks of potable water, storm water, sewers and natural gas, and by the rebuild of the neighborhood. The vulnerability index was estimated on the basis of the mentioned basic urban services. The district "Puente Alsina", is an establishment with more than 300 families (according to the census made in March of 2011) located in the City of Valentin Alsina, of the Municipality of Lanus, the Buenos Aires Province. The environmental problems are put in evidence by the contamination of ground, air and water. This area receives pollution from Matanza Riachuelo system since it is located in the floodplain, whose levels of contamination generate a potential risk for the health of the inhabitants, added to the environmental liabilities originated by a distillery in disuse next to the zone under study. These environmental liabilities are product of tanks of hydrocarbon storage that are present in the field of the industry, which is destined to logistic activities. Actions to implement in order to improve the indices of vulnerability under study are: 1- Territorial ordering, 2- soil remediation, 3- Remediate coastal area and the towpath. In order to diminish the impact environmental the community will participate in working programs. Gradually, reduced groups of the population will be provisory relocated, in functional units in the contiguous land belonging to the Belgrano Railroad logistic and storage area which is in disuse. Main collectors and water and natural gas will be installed in a first instance. After system of dumps to prevent foods different networks will be installed in modular stages and houses will be rebuilt. Finished each stage, families will be reinserted to their house in a legally way and they will refund with work in another site or paying a mortgage to be the owners.

**TP173 Risks Associated to the Lack of Urban Services in El Campanario Neighborhood** M. Calle, E. Villaverde, A.N. Monzon, National University of Lanus; S.O Demichelis, Urban Environmental Management / Natural Sciences Faculty, National University of Lanus (UNLa) / Urban Environmental Management-Department of Productive Development and Technology. The aim of the present study is diminish risk by the amelioration of the basic urban services at the neighborhood "El Campanario", located in Llavallol, Municipality of Lomas de Zamora, Buenos Aires Province. Basic urban services included are the management of solid wastes, sewages and storm water drainages and systems of distribution for water, electrical power and natural gas. This urbanization has approximately six hundred families, distributed in seventeen buildings. The existing services and infrastructure has a great deterioration degree, and there is absence of potable water service and rubbish collection. These facts have an impact in the health of the local population which is demonstrated by the predominance of diseases such as hepatitis, diarrhea, anemia, and others. The situation of the building structures, added to overcrowding problems



aggravates the situation and difficulties the planning of the services provision. In order fulfill the requirements to decrease risk sources of this neighborhood some plans of action are proposed: 1-Rubbish Management will include containers to collect waste and organize waste transport, treatment and recycling of certain products which will accorded with the community. 2-Potable water distribution: will include the design of a network, in order to eliminate the local source of supply which is a well with water enriched with pollutants such as nitrates and other inorganic salts. 3-Natural gas network: It is proposed to renew the gas networks due to antiquity and absence of maintenance which result in the deterioration of existent system; therefore numerous escapes risks the resident population. 4-Electrical power: to revise all networks in order to ameliorate the existent network and to eliminate the clandestine and create new connections. 5-Sewages and water-drainages: to replace the existing collection systems in order to adapt them to this growing population.

**TP174 US EPA Bisphenol A alternatives in thermal paper: Substitution options** J. Rhoades, M. Kawa, SRC, Inc.; C. Baier-Anderson, US Environmental Protection Agency / Design for the Environment; E.T. Lavoie, US Environmental Protection Agency / Design for the Environment Program, Environmental Protection Agency / Office of Pollution Prevention and Toxics, U.S. Environmental Protection Agency / Toxicologist; J. Tunkel, SRC, Inc. / Chemical, Biologica, Environmental Center. The U.S. Environmental Protection Agency (US EPA) Design for Environment (DfE) Program undertook a chemical alternatives assessment for the use of bisphenol A (BPA) in thermal paper, including cash register receipts, as part of the Action Plan for BPA in March 2010. The alternatives assessment was done via a multi-stakeholder partnership that identified 19 functional alternatives to BPA. The hazard assessment for BPA and the alternatives used the DfE hazard evaluation criteria to assign hazard designations for human health toxicity, ecological toxicity and environmental fate endpoints. Some alternatives were well characterized for all endpoints. Other alternatives were poorly characterized, wherein analog data, predictive models, structural alerts and expert judgment were used to make hazard designations for data gaps. This assessment also focused on the combined use of physical/chemical property and ecotoxicity models for assigning hazard designations for acute and chronic endpoints. As a result of the structural similarity, human health, ecological toxicity and fate characteristics are very similar. There are, however, several chemicals that exhibit different hazard designations based on the presence of specific functional groups. Effective hazard assessment approaches, coupled with decision-making protocols that are practical tools for businesses to use in materials selections, will contribute to more sustainable product development when human health or ecological toxicity concerns exist. The resulting hazard profiles should be of value to manufacturers making substitution decisions and facilitate reductions in potential human health impacts.

**TP175 Use of Exposure Point Concentrations for Contaminated Groundwater Throughout the CERCLA Process** A. Aly, Intera. The use of exposure point concentration (EPC) in the baseline risk assessment is well described in the EPA and other guidance. Similarly RCRA compliance guidelines from several states and the EPA guidance on attainment of groundwater cleanup levels are also reasonably described. However, throughout the CERCLA process and during the remedial investigation evaluation of baseline conditions and the feasibility study evaluation of remedial alternatives, no clear guidance is provided on the use of the EPC. An approach is developed to provide a clear transition from the baseline risk assessment, to remedial alternatives evaluation, monitoring requirements, and finally demonstration of cleanup attainment. The approach uses different estimates of the EPC throughout the CERCLA process to allow a continuous and smooth transition from the baseline risk assessment to the demonstration of cleanup attainment.

**TP176 A time-course analysis of effects of the steroidogenesis inhibitor ketoconazole on the hypothalamic-pituitary-gonadal axis of fathead minnows** J. Cavallin, U.S. EPA, ORISE / National Health and Environmental Effects Research Laboratory; E. Durhan, US EPA / Mid-Continent Ecology Division, US-EPA / Research Chemist; K. Jensen, M. Kahl, U.S. EPA / National Health and Environmental Effects Research Laboratory; E. Makynen, US EPA / Mid-Continent Ecology Division; L.M. Thomas, US Fish and Wildlife Service / Mid-Continent Ecology Division; L.C. Wehmas, Oregon St. University / Mid-Continent Ecology Division; D.L. Villeneuve, U.S. EPA / National Health and Environmental Effects Research

Laboratory, U.S. EPA; G.T. Ankley, US EPA / Office of Research and Development, National Health and Environmental Effects Research Laboratory, Mid-Continent Ecology Division, U.S. EPA / National Health and Environmental Effects Research Laboratory. The objective of this study was to evaluate temporal effects of the model steroidogenesis inhibitor ketoconazole (KTC) on aspects of reproductive endocrine function controlled by the hypothalamic-pituitary-gonadal (HPG) axis in the fathead minnow (*Pimephales promelas*). Ketoconazole inhibits the activity of two cytochrome P450s (CYPs) key to sex steroid production in vertebrates, CYP11a and CYP17. In these studies, sexually-mature fish were exposed to KTC (30 or 300 µg/L) for up to 8 d, following which animals were allowed to recover in clean water. Samples were collected after 1, 4 and 8 d of exposure, and after 1, 8 and 16 d of recovery. A shorter-term time-course experiment was conducted in which females were sampled on seven occasions during a 12-h KTC exposure. Ketoconazole depressed *ex vivo* gonadal synthesis of testosterone (T) in both sexes, and 17β-estradiol (E2) in females during exposure and recovery phases of the time-course studies. Effects on *ex vivo* steroidogenesis in females occurred within as little as 1 h of exposure. Plasma concentrations of T in males and E2 in females also were depressed early in the KTC exposure, but those decreases did not persist to the same degree as observed for the *ex vivo* effects. In females, after decreases within 12 h, plasma E2 concentrations were similar to (or greater than) controls at 24 h of exposure, while in males, plasma T returned to levels comparable to controls within 1 d of cessation of KTC exposure. The discrepancy between the *ex vivo* and *in vivo* data at later stages in the test is consistent with some type of compensatory response to KTC in fish. However, we were unable to ascertain the mechanistic basis for such a response. Although a number of genes related to steroid synthesis were up-regulated in male and female gonads during the exposure and early recovery phases of the experiment, this did not seem to account for the resurgence in plasma steroid concentrations in KTC-exposed fish. Further studies focused on metabolism and clearance of steroids might lend insights as to the effects of KTC on plasma steroid concentrations. Overall, our results demonstrate the complex, temporally-dynamic nature of the vertebrate HPG system in response to chemical stressors.

**TP177 Accounting for Intended Use Application in Characterizing Contributions of D5 to Aquatic Loadings Following Marketed Personal Care Product Use** B. Montemayor, Canadian Cosmetic, Toiletry and Fragrance Association, Canadian Cosmetic, Toiletry and Fragrance Association / Toiletry and Fragrance Association; B. Price, Procter & Gamble; R. van Egmond, Unilever Research Laboratory Colworth / SEAC. Decamethylcyclotrisiloxane, commonly known as D5 (cyclotrisiloxane) has a wide application of use across a multitude of personal care product categories. The relative volatility of D5 is one of the key properties attributed to this substance that provide for the derived performance benefits attributed to the use of this raw material in personal care formulations. On this basis, rapid evaporative loss following use of many products comprising D5 is expected following typical use application and corresponding wear time. Studies were conducted on three key product categories containing D5 (antiperspirants, skin care products and hair care products) to investigate the amount of D5 that may be destined to go down the drain following simulated typical personal care use scenarios. Marketed antiperspirants and skin care products were applied to human subjects and hair care products were applied to human hair tresses and subsequently rinsed off at designated time points. Wash water was collected at 0, 8 and 24 hours (antiperspirant and hair care analysis) and additionally at 4 hours (skin care analysis) post product application and samples analyzed by isotope dilution headspace gas chromatography/mass spectrometry (GC/MS) to quantify the concentration of D5 destined to be available to go down the drain in captured wash water. It is demonstrated that only a very small fraction of the D5 in products (less than 1%, 0.3 % and 0.02% for antiperspirants, skin care and hair care products, respectively) is available to go down the drain from 'leave-on' type personal care products following typical conditions of use.

**TP178 Acute effects of β-naphthoflavone on swim performance, cardiorespiratory function, and energy stores in adult zebrafish (*Danio rerio*)** C.J. Gerner, University of Saskatchewan / Toxicology Graduate Program, University of Saskatchewan; L. Weber, University of Saskatchewan / Toxicology Research Centre. The class of chemicals known as polycyclic aromatic hydrocarbons (PAHs) are known agonists of the aryl hydrocarbon receptor (AHR). They are also contaminants of rivers, lakes, and marine shorelines, making fish a primary target species, but acute adult fish toxicity is thought

to be minimal or absent. In the present study adult zebrafish (*Danio rerio*) were aqueously exposed to solvent control (DMSO) or three increasing concentrations of the commonly used model PAH  $\beta$ -naphthoflavone (BNF; 0.1, 10, and 1000  $\mu\text{g/L}$ ) for a 48 hour period. Following exposure fish were subjected to swimming tests with concurrent oxygen consumption measurement ( $n=10$  fish/treatment) or echocardiography to determine cardiac function ( $n=10$  fish/treatment). Oxygen consumption ( $\text{MO}_2$ ) was increased at all exposure concentrations compared to control, reaching statistical significance at the second highest BNF exposure at a swim speed of 23.1 cm/s ( $p < 0.01$  in Fisher's LSD test after two-way ANOVA).  $\text{MO}_2$  at zero water velocity was positively correlated with ventricular volume at diastole ( $r=0.957$ ) and ejection fraction ( $r=0.859$ ), but negatively correlated with acceleration of blood through the ventricle ( $r=-0.988$ ) in resting fish. In contrast, BNF had no significant effect on  $U_{\text{crit}}$ , tissue triglyceride, or glycogen concentrations. The effect of BNF on  $\text{MO}_2$  is likely to be physiologically important given that fish have a critical need for adequate oxygen to fuel aerobic activities such as swimming. Future studies will involve examining the effects of the more toxic and environmentally relevant PAH, benzo[a]pyrene, on fish cardiorespiratory function and swim performance.

**TP179 Acute to Chronic Ratios of Pharmaceuticals to Support Environmental Risk Assessments** J. Small, Merck & Co., Inc. / Global Safety and the Environment, Merck & Co., Inc.; V.J. D'Aco, Quantum Management Group, Inc.; D.J. Caldwell, Johnson & Johnson; L.A. Constantine, Pfizer Global Research and Development / Pharmacokinetics, Dynamics & Metabolism, Pfizer, Inc. / Pharmacokinetics, Dynamics and Metabolism, Pfizer, Inc. / Chemical Research & Development; T. Davidson, Bristol-Myers Squibb; D. Dolan, Amgen; J.F. Ericson, Pfizer Inc / Environmental Sciences / PDM; R. Hannah, GlaxoSmithKline / CEHS – RS 1105, GlaxoSmithKline / Sustainability & Environment; R.D. Meyerhoff, Eli Lilly and Company / Lrl- Health, Safety And Environmental; J. Ryan, GlaxoSmithKline; G. Panter, AstraZeneca UK Limited / Brixham Environmental Lab.; N. Parke, Eli Lilly and Company; K. Quinlan, J&J PRD, LLC / Environmental Engineer; J.G. Tell, Merck & Company, Inc. / Global Safety & the Environment, Merck & Company, Inc. / Safety and Environment Department; P. Wilson, Sanofi-Aventis U.S., Inc. / HSE. The PhRMA PIE Task Force has formed a technical team to evaluate the relationship between acute  $\text{EC}_{50}$  and chronic NOEC ratios for a broad range of active pharmaceutical ingredients (APIs) in various therapeutic areas and with diverse modes of action. The two main research areas of interest are to determine if current default safety factors applied in environmental risk assessments are adequate, and to evaluate the overall toxicity of pharmaceuticals in water. The ten member companies of this task force provided internal, blinded, acute and chronic daphnia and fish data for approximately 90 compounds, supplemented with ecotoxicity data for other APIs abstracted from the peer-reviewed literature, in order to compare these various endpoints. All compounds were classified according to the American Hospital Formulary Service (AHFS) classification scheme at the primary level. Compounds were grouped into like classes and trends of toxicity and acute : chronic ratios were assessed. In the majority of cases, acute : chronic ratios remained below 1000, consistent with current tiered regulatory schemes in the United States.

**TP180 Antimicrobials, Antimicrobial Resistance and New Risk Assessment Tools** R.U. Halden, Arizona State University / Center for Environmental Security, The Biodesign Institute at Arizona State University. Antimicrobial agents are recognized as lifesaving products of applied chemistry. Yet, their extensive use in disposable household products is known to cause widespread environmental contamination of aquatic and terrestrial environments. This paper provides an overview of the current state of knowledge concerning the fate and behavior in the environment of two widely used persistent, bioaccumulative and toxic antimicrobial agents: triclosan (TCS) and triclocarban (TCC). Known environmental health and safety concerns are discussed along with opportunities to meet public health objectives in an environmentally responsible and sustainable fashion. This paper can serve as an example of how environmental monitoring at municipal wastewater treatment plants can inform public policy and drive the development of regulations protective of human health and the environment.

**TP181 Assessing the impacts of the xenoestrogens p-nonylphenol and 17 $\alpha$ -ethinylestradiol on larval horseshoe crabs** E. Maung-Douglass, University of Delaware; D.C. Miller, University of Delaware / College of Earth,

Ocean and Environment. *Limulus polyphemus* is one of four species of horseshoe crab and the only one of those species to reside in North America. Horseshoe crabs spend the first few weeks of their lives developing in beach sediments where they are susceptible to consumption by migratory shore birds and exposure to environmental contaminants. Horseshoe crabs are of great ecological, economic and biomedical importance, and their populations are declining. Thus, it is vital that we better understand the impacts of common contaminants, like xenobiotics, on the larval development of this species. Horseshoe crab embryos were sampled from a coastal bay within Delaware. Compound exposure experiments were performed on these embryos and monitored for larval development. Embryos were exposed to individual compounds (p-nonylphenol and 17 $\alpha$ -ethinylestradiol) at levels ranging from 0-1000 mg/L for periods up to 26 days. Significant delays in development were detected in the 1, 10, 100 and 1000 mg/L nonylphenol treatments, but not in the 17 $\alpha$ -ethinylestradiol treatments. In a compound combination experiment, larval development was significantly delayed at doses of 1000 mg/L NP + 100 mg/L EE2. Individuals from the 100 mg/L NP + 100 mg/L EE2 and 1000 mg/L NP + 100 mg/L EE2 treatments had significant delays in development. These treatments were developmentally delayed by as much as one week when compared with the control group. In addition to developmental delays, morphological deformities also occurred in 4.5-18% of individuals in the 100 mg/L NP + 100 mg/L EE2 treatment. Our findings have implications for environmental conservation and management for horseshoe crabs nesting sites within close proximity to sewage treatment plant outfall, a source of xenobiotics.

**TP182 Assessment of Species Specific Sensitivity of North American Fishes to Disruptors of Steroidogenesis In vitro** S. Beitel, J. Doering, E. Higley, S. Patterson, H. Prodhahl, University of Saskatchewan / Toxicology Centre; S.B. Wiseman, University of Saskatchewan / Toxicology Centre, University of Saskatchewan / Post-Doctoral Fellow; M. Hecker, University of Saskatchewan / Toxicology Centre. There is increasing concern regarding the release of natural and synthetic chemicals into the environment that can interfere with the endocrine system of aquatic organisms. Fishes are particularly at risk of exposure to these endocrine disrupting chemicals (EDCs) as many of these compounds are directly released into the aquatic environment through industrial and communal effluents, and agricultural runoff. To enable objective risk assessment of such chemicals, it is critical to identify the sensitivity of species native to the environments in question. In this context, little is known regarding the sensitivity of fishes native to northern ecosystems to EDCs, in particular disruptors of steroidogenesis. From an ethical, logistical (i.e. sufficient large sample size) and economical perspective, use of whole organisms is becoming increasingly questionable. In context with the 3R concept (reduce, replace, refine) there is need for alternative methods reducing the use of large number of animals in ecotoxicology testing. Therefore, the aim of this study was to develop an *in vitro* gonadal explant assay that enables the assessment of EDC effects on sex-steroid production in wild fish species. Mature male and female northern pike (*Esox lucius*), walleye (*Sander vitreus*) and white sucker (*Catostomus commersoni*) were sampled from Lake Diefenbaker, Saskatchewan, Canada. Gonadal tissues were excised and exposed for 24 hours in 24 well plates containing media dosed with a model inducer (forskolin) and inhibitor (prochloraz) of steroidogenesis. Concentrations tested were 0.3, 1.0, 3.0, 10.0, 30.0  $\mu\text{M}$  of forskolin and 0.03, 0.1, 0.3, 1.0, 3.0  $\mu\text{M}$  of prochloraz. Sex steroid concentrations present in media were quantified using HPLC-MS/MS. Preliminary data showed female white sucker and walleye having the greatest sensitivity to prochloraz and forskolin, respectively. Exposure to prochloraz resulted in a dose dependant increase in testosterone and decrease in estradiol, while exposure to forskolin resulted in a dose dependant increase in both testosterone and estradiol. Based on this preliminary data, this *in vitro* approach represents a promising tool for the assessment of species-specific responses to disruptors of sex steroidogenesis. Further characterization of molecular and biochemical endpoints in the gonads and liver, and comparisons to *in vivo* effects are ongoing in an effort to validate these *in vitro* methods as a predictive ecotoxicological tool.

**TP183 Binding affinity of antidepressants to the serotonin reuptake transporter as a predictor of adverse behavioral effects in fish** L. Sweet, Clemson University / Institute of Environmental Toxicology; J.H. Bisesi, Clemson University / Institute of Environmental Toxicology (CU-ENTOX), University of Florida / Department of Environmental and Global Health; S.J. Klaine, Clemson University / Institute of Environmental Toxicology

(CU-ENTOX), Clemson Institute of Environmental Toxicology / Institute of Environmental Toxicology (ENTOX), Clemson University / Institute of Environmental Toxicology (ENTOX). Widespread use of antidepressants and their incomplete removal during wastewater treatment has resulted in their increased detection in many surface waters. Our previous research has shown that exposure to antidepressants that act as selective serotonin reuptake inhibitors (SSRI) decreases brain serotonin levels and increases the time it takes for a predator (*Morone saxatilis* x *M. chrysops*, hybrid striped bass) to capture its prey (*Pimephales promelas*, fathead minnow). Further, this relationship is quantitative and exposure to more than one SSRI results in additive effects. However, exposure to bupropion, a norepinephrine and dopamine reuptake inhibitor that does not target the serotonin system, did not affect predation behavior. These results have led to the hypothesis that there is a mechanistic link between SSRI antidepressant exposure, brain serotonin levels, and alterations in predation behavior in fish. The objective of the current study was to quantify the relationship between the binding affinity of SSRI antidepressants to their target receptor, the serotonin reuptake transporter, and adverse behavioral responses in fish. A zebrafish serotonin reuptake transporter plasmid construct was overexpressed in mammalian cell lines to produce sufficient protein for receptor binding affinity assays. The binding affinities ( $K_i$ ) of fluoxetine, venlafaxine, and bupropion were measured using radio-ligand binding assays and correlated with brain serotonin concentrations and observed behavioral effects from hybrid striped bass exposed to the respective antidepressants. Furthermore, the binding affinity of other selective serotonin reuptake inhibitor antidepressants were investigated to provide *in vitro* predictions of brain chemistry and behavioral alterations observed *in vivo*. Results from this study will provide a valuable tool for predicting the effects of exposures to multiple antidepressants on subtle behaviors that directly influence ecological fitness and population survival; but are not captured using traditional toxicity testing endpoints.

**TP184 Chronic toxicity of amoxicillin, neomycin, and tylosin and their potential ecological risks in water** S. Lee, Seoul National University / School of Public Health; S. Lee, Seoul National University / School of Public Health, Seoul National University; C. Kim, Seoul National University / School of Public Health; X. Liu, Seoul National University, Seoul National University / School of Public Health; Y. Kho, Eulji University / School of Human & Environmental Sciences; P. Kim, National Institute of Environmental Research; K. Choi, Seoul National University / School of Public Health. Pharmaceuticals are of concern because of their frequent detection in aquatic environment and potential ecological effects. However only limited information is known about ecotoxicological effects of chronic exposure to pharmaceuticals on aquatic organism. Chronic toxicity of three commonly detected pharmaceuticals, i.e., amoxicillin, neomycin, and tylosin was evaluated using an algae, two waterflea and two fish species. We conducted long term exposure tests using teleost, *Oryzias latipes* (40 d) or *Danio rerio* (32 d), two cladocerans, *Daphnia magna* (21 d) and *Moina macrocopa* (7 d), and freshwater algae, *Pseudokirchneriella subcapitata* (72 hr), following OECD guideline and literature. Based on the toxicity values, the predicted no effect concentrations (PNECs) were derived and hazard quotients were estimated. No observed effect concentrations (NOECs) of Amoxicillin were observed at 213.14 mg/L, 33.3 mg/L, and 1.11 mg/L for *P. subcapitata*, *D. magna*, and *O. latipes*, respectively. NOECs derived by Neomycin exposure were at 4.6 mg/L, 0.19 mg/L, and 0.1 mg/L for *P. subcapitata*, *D. magna*, and *O. latipes*. NOECs of tylosin were at 5 mg/L, 100 mg/L for *D. magna* and *D. rerio*, respectively. PNECs derived based on available toxicity values were \*, \*, and \* for amoxicillin, neomycin, and tylosin, respectively. Hazard quotients (HQs) estimated for amoxicillin, neomycin and tylosin were 0.0001, 0.3133 and 0.0006, respectively, based on mean detected concentrations in surface water of Korea. Our results showed that these chemicals are not of concern at the current level of occurrences. However the HQ of neomycin suggests this pharmaceutical could be of potential problem in extreme exposure situations e.g., near WWTP outfalls.

**TP185 Comparative pharmacology and toxicology: Interactive toxicity of diphenhydramine and acetylcholinesterase inhibitors in fish and invertebrate models** L.A. Kristofco, Baylor University / Department of Environmental Science, Center for Reservoir and Aquatic Systems Research; B. Du, Baylor University / The Institute of Ecological, Earth, Environmental Sciences, Baylor University / Department of Chemistry and Biochemistry; Department of Environmental Science, Baylor, Baylor University / The Institute of Ecological, Earth, Environmental Sciences, Baylor; K. Chambliss,

Baylor University / Department of chemistry and biochemistry; J. Berninger, Baylor University / Department of Environmental Science; B.W. Brooks, Baylor University / Environmental Science and Biomedical Studies, Baylor University / Department of Environmental Science. In rapidly urbanizing regions of the southwestern US, instream flows are increasingly dominated by discharges from wastewater treatment plants. Future population growth and climate change projections for Texas indicate that base flows of many river systems will depend on these effluents, which contain a complex mixture of contaminants. Interactive effects of pesticides and pharmaceuticals, however, are poorly understood. The objective of this study was to examine the influence of mixtures of the antihistamine, diphenhydramine (DPH), and acetylcholinesterase inhibiting (AChEI) insecticides to fish and invertebrates. Though DPH inhibits the H1 receptor, eliciting antihistamine responses, in mammals DPH also inhibits serotonin uptake at the serotonin reuptake transporter and functions as a competitive antagonist for the acetylcholine receptor (AChR). In fact, due to AChR inhibition DPH has been suggested as an emergency medicine treatment for AChE poisoning. Based on evolutionary conservation of cholinergic neurotransmissions among animals, we hypothesized that the mammalian pharmacology and toxicology data for AChEI and DPH mixtures will predict the nature of chemical mixture toxicity to model aquatic organisms. Toxicity experiments generally followed experimental designs recommended by standardized EPA methods. We also employed the Fish Embryo Toxicity (FET) test method, which represents an alternative to traditional acute fish toxicity testing, and supports the European Union's (EU's) REACH (Registration, Evaluation, Authorization and Restriction of Chemicals) requirement to reduce the number of vertebrate organisms utilized in chemical safety assessments. The acute and chronic toxicity of invertebrate *Daphnia magna*, and the acute toxicity and development defects of *Danio rerio* (zebrafish) embryos to single compounds and AChEI/DPH mixtures, were evaluated to define interactive effects. Toxicity thresholds of individual chemicals were consistent with previous studies from our group and other labs. Contrary to our hypothesis, cladoceran responses did not indicate protective effects of diphenhydramine during coexposures with the AChEI diazinon.

**TP186 Development of a fluorescence polarization, competitive receptor-ligand binding assay of thyroid hormone disruption by PBDEs and PCBs in zebrafish** K. Stencel, Duke University / Nicholas School of the Environment; W. Dong, Nicholas School of the Environment, Duke University, Durham, NC / Researcher; A. Save, Duke University; H.M. Stapleton, Duke University / Nicholas School of the Environment; J. Zheng, University of Delaware / Department of Chemistry and Biochemistry; L. Ferguson, Duke University / Department of Civil and Environmental Engineering, Duke University / Nicholas School of the Environment, Duke University / Department of Civil & Environmental Engineering, Pratt School of Engineering / Department of Civil & Environmental Engineering. Thyroid hormones act to regulate important biological pathways in development as well as metabolism. Recent work shows that many chemicals used as flame-retardants, including polybrominated diphenylethers (PBDEs) and their metabolites may act as endocrine disruptors by altering the thyroid hormone system. A sensitive, *in vitro* assay using recombinant zebrafish nuclear thyroid hormone receptor beta (TR $\beta$ ) was developed for assessing structure-activity relationships of potential thyroid-disrupting chemicals, namely PBDEs, PCBs, and their derivatives. The assay was based on the fluorescence polarization technique, which directly measures binding of a specially-synthesized, fluorescein-labeled thyroid hormone analogue to recombinant TR $\beta$  ligand binding domain at excitation and emission wavelengths of 485 nm and 535 nm, respectively. The affinity of the fluorescent probe for TR $\beta$  was measured by saturation-binding experiments and was in the nanomolar range. Subsequently, competitive binding assays for the thyroid hormone, T<sub>3</sub>, and halogenated and hydroxylated diphenyl ethers were performed to assess binding and thus biological activity to zebrafish TR $\beta$ . The development of this rapid, homogeneous, and sensitive screening method for thyroid active compounds in our laboratory has enabled screening of complex environmental samples for thyroid-disrupting activity.

**TP187 Development of a model to estimate the human pharmaceuticals emission into surface water and assessment of the model uncertainty** E. Han, D. Lee, Environmental planning Institute, Graduate school of environmental studies, Seoul National University. Accurate prediction of emission is critical to assessing and managing exposure of pharmaceuticals in freshwater, particularly because extensive monitoring data are often lacking.



In the present work, an emission prediction model is presented with the uncertainties associated with the model prediction of human pharmaceuticals discharged into typical Korean river environment. The model covers the stages of pharmaceutical life cycle posterior to domestic production and import, including distribution, consumption, disposal, and waste treatment. A total of 27 parameters are used in the model, which can be divided into five groups (i.e. initial input into life cycle, fate properties, distribution ratio among the parties in supply chain, patient's behavior on drug administration and disposal, and waste treatment process train). As exact values were not known for eight variables among the 27 variables, uncertainty analysis of the model estimation was conducted by using Monte-Carlo calculations. A uniform distribution of the value with practically possible full range was assigned initially to each of the eight variables. Given the distributions, the emission to Korean river was estimated to range from zero to 85% of the total quantity produced and imported. It was also found that the excretion rate (ER) and the removal rate in sewage treatment plant (RR.stp) (both are the pharmaceutical-dependent fate properties) most sensitively influence the emission estimates. Once ER and RR.stp are fixed at any value, the variables of the highest sensitivity were the administration rate of outpatient and the return rate to the Take-back program (both are the patient's behavior dependent parameters). When coupled with an environmental quality model, the emission estimate with its uncertainty for 4 test pharmaceuticals provided results that are in good agreement with the measured concentrations in water. The model's capability to quantitatively estimate the emission and its uncertainty/sensitivity for individual pharmaceuticals is expected to assist in finding an effective management strategy for pharmaceuticals emission into surface water.

**TP188 Effects of 17 $\beta$ -Estradiol exposure on gamete development and viability in freshwater Unionids** D. Sovic, Ohio State University; R. Lanno, Ohio State University / Department of Evolution, Ecology, and Organismal Biology. Evidence of a functional role for vertebrate steroids has been demonstrated in a number of invertebrate species, including several molluscs. This knowledge, in turn, has generated interest and research into the possibility of invertebrate endocrine disruption due to exposure to both exogenous natural steroid hormones and xenobiotics that have the potential to mimic the action of these compounds. Exposure to the natural vertebrate estrogen, 17 $\beta$ -estradiol (E2), for example, has been shown to induce accelerated gamete development in multiple mollusc species. Little information is available, however, for the freshwater Unionid bivalves, a group for which much concern over declining populations and loss of species currently exists. In order to investigate the effects of E2 exposure on gamete maturation and viability in freshwater Unionids, two species, *Lampsilis cardium* and *Elliptio complanata*, were dosed at one of three exposure levels (5400, 5.4, and 0.0054 ng) via direct injection into the foot. All mussels were held in raceways fed by a constant flow of river water throughout the duration of the study. Exposures were carried out across multiple durations and at different points in the reproductive cycle. Following each exposure period, biopsies containing developing gametes were collected from each individual, and either ova development, as measured by ova diameter, or sperm maturation and motility were assessed and compared with controls.

**TP189 Effects of non-steroidal anti-inflammatory drugs on hormone production and gene expression in hypothalamic-pituitary-gonad axis of zebrafish** K. Ji, University of Saskatchewan / Biomedical Veterinary Sciences and Toxicology Centre, University of Saskatchewan; X. Liu, S. Lee, S. Kang, Seoul National University; J.P. Giesy, University of Saskatchewan; K. Choi, Seoul National University. Non-steroidal anti-inflammatory drugs (NSAIDs) have shown estrogenic activity *in vitro* and *in vivo*, but the mechanism of this activity is not fully understood. In this study, plasma hormone levels as well as expressions of several mRNAs along the hypothalamic-pituitary-gonad (HPG) axis were measured in adult male and female zebrafish after exposure to 0, 0.01, 0.1, and 1 mg/L of acetylsalicylic acid, diclofenac, ibuprofen, mefenamic acid, or naproxen for 14 days. Concentrations of 17 $\beta$ -estradiol (E2) in plasma were significantly increased in fish of both sexes exposed to 0.01 or 0.1 mg/L ibuprofen and mefenamic acid. Concentrations of testosterone (T) in females exposed to ibuprofen and mefenamic acid were also significantly greater than in controls, while lesser concentrations were observed in males. The ratio of E2/T and the expression of *CYP19a* mRNA in gonad was significantly up-regulated in males and females, providing evidence of increased conversion of T to E2. In females exposed to five NSAIDs, concentrations of follicle-stimulating hormone

(FSH) and luteinizing hormone (LH) as well as the transcription levels of *FSH $\beta$* , *LH $\beta$* , *FSHR* and *LHR* were significantly greater than in controls, suggesting the promotion of gametogenesis and oocyte maturation. In males, the mRNA expressions of *FSH $\beta$*  and *LH $\beta$*  were significantly down-regulated along with a decrease of FSH and LH, suggesting a delay in spermatogenesis. The results of this study show exposure to NSAIDs altered the expression of regulatory genes and hormones of the HPG axis in zebrafish, and the responses were sex dependent.

**TP190 Endocrine disruption effect of chronic exposure to mefenamic acid on aquatic organisms *Daphnia magna*, *Moina macrocopa* and *Danio rerio*** H. Jung; S. Kang, S. Kim, K. Ji, K. Choi, Seoul National University. Pharmaceuticals are trace contaminants of growing importance in aquatic ecosystem due to their physiologically active nature and their potential for impacts on non-target species. Mefenamic acid (MFA, CAS No. 50-78-2) is a non-steroidal anti-inflammatory drug (NSAIDs), which has been widely used for analgesic, antipyretic and anti-inflammatory purposes. We evaluated the acute and chronic toxicity of mefenamic acid employing several aquatic organisms, including two cladocerans, *Daphnia magna* and *Moina macrocopa*, and a teleost, *Danio rerio*. The 48 h acute median effective concentration (EC<sub>50</sub>) of *D. magna* was determined at 17.16 mg/L. In 21 d chronic toxicity test, *D. magna* showed significant changes in reproduction related characteristics after the exposure. In the early life stage test using *D. rerio*, there was a significant decrease of larval survival at as low as 1 mg/L of mefenamic acid. In addition, vitellogenin gene expression and VTG protein level were reduced at the highest experimental concentration of mefenamic acid, 1 mg/L. The result of this study indicates that this pharmaceutical may have a potential effect on survival, reproduction and growth of the aquatic organisms, but the effect concentrations are a few orders of magnitude greater than those occurring in the ambient water environment.

**TP191 Establishing Thresholds for Persistent Pollutants in Municipal Effluent** B.K. Hope, CH2M Hill. In 2007, the Oregon Legislature enacted Senate Bill 737 (SB 737) which required the Oregon Department of Environmental Quality (ODEQ) to develop a list of persistent pollutants that could adversely impact waters of the state. ODEQ developed and implemented a selection process consistent with this legislation and, in October 2009, produced a list of 118 persistent pollutants. SB 737 also required Oregon's 52 largest municipal waste water treatment plants and water pollution control facilities to prepare reduction plans for persistent pollutants detected in effluent above a Plan Initiation Level (PIL [formerly referred to as a "trigger level"]). Per the SB 737 legislation, a PIL is a maximum contaminant level (MCL) established by U.S. EPA National Primary Drinking Water regulations or, for those pollutants without an MCL, a level established in Oregon administrative rule. Of the 118 listed pollutants, only 33 had an MCL, requiring ODEQ to develop and implement a process to select PILs for the remaining 85 pollutants. PILs for chemicals without an MCL were selected (or calculated with existing data) from national or international government documents or the scientific literature, using a hierarchical process. Because SB 737 included, in addition to the required use a MCL if available, a broad mandate to protect human health, fish, wildlife, and aquatic species, selected PILs had to represent a chemical concentration below which: (a) aquatic life is not expected to experience adverse effects when directly and chronically exposed to chemicals originating in effluent or (b) humans are not expected to experience cancer or non-cancer effects when directly exposed to those same chemicals in effluent. PILs were ultimately established in administrative rule by the Oregon Environmental Quality Commission in June 2010. This presentation will describe and discuss hierarchical process used to select those PILs.

**TP192 Estrogenic effect of natural water from Itapecuru River, Maranhao, Brazil, on males of *Danio rerio*** R.T. Verbrinnen, Sao Paulo University, University of Sao Paulo / Instituto de Quimica de Sao Carlos / Departamento de Quimica e Fisica Molecular; R.N. Padovan, University of Sao Paulo / Instituto de Quimica de Sao Carlos / Departamento de Quimica e Fisica Molecular; E.M. Vieira, Sao Paulo University / Molecular Physics Chemistry Department, University of Sao Paulo / Instituto de Quimica de Sao Carlos / Departamento de Quimica e Fisica Molecular; R.L. Luvizotto-Santos, Federal University of Maranhao / Departamento de Oceanografia e Limnologia. Several studies notice the occurrence of substances capable to disrupt the endocrine system, in rivers from different regions of the planet. Pollutants with such characteristic belong to distinct chemical groups, being,

for instance, estrogens, pesticides, plastic additives, polychlorinated biphenyl and others. In the majority cases, they reach the rivers through sewage. The fish feminization is a disrupt effect highlighted in some cases. The Itapecuru River is the water source for about 50% (=507,419 habitants) of the population from Sao Luis, the capital of the State of Maranhao, Brazil, and at least other 10 cities along the river. On its whole watershed, the river is reached by the sewage from 52 cities, released by an estimated population of 3 million people. The aim of this study was to investigate the occurrence of endocrine disruption in fishes exposed to the Itapecuru River natural water, by quantification of the biomarker vitelogenin (VTG). From March 23 to April 13, 2011, a chronic assay was run during 21 days with a 50% daily water renovation, natural photoperiod and feeding twice a day with commercial food. Adults zebrafish (*Danio rerio*) were divided among three groups and distributed equally on 9 aquariums of capacity of 10 liters, maintained under constant aeration. Each aquarium received 5 female fish and 5 male fish. The aquariums were filled up with 100% river water, with 100% reconstituted water (control) and with a mix of 50% of each water. VTG was analyzed using a commercial ELISA kit (biosense Laboratories). In comparison to the control group, the Kruskal Wallis test ( $p < 0.05$ ) demonstrated a significant increase ( $H = 15.08824$  and  $P = 0.0001$ ) of the VTG level in male fishes from the 100% river water group. The results suggest the presence of estrogenic effect substances in a quantity enough to promote biochemistry alterations associated to the fish reproduction.

**TP193 Exposure to POPs and potential health effects among matching mother and fetus pairs** S. Kang, Seoul National University / 1 School of Public Health; S. Kim, School of Public Health, Seoul National University / Environmental Health, Seoul National University / Graduate School of Public Health; S. Kim, Seoul National University / School of Public Health; H. Moon, S. Lee, Hanyang University / College of Science and Technology; J. Lee, G. Choi, Soonchunhyang University Hospital / Department of Environmental Health Sciences; S. Kim, S. Choi, Hallym University Medical Center / Department of Obstetrics and Gynecology; H. Kim, Korea University Ansan Hospital / Department of Obstetrics and Gynecology; J. Park, Soonchunhyang University / Department of Obstetrics and Gynecology; S. Kim, Jeju National University / School of Medicine; H. Nam, Korea Food and Drug Administration / National Institute of Food and Drug Safety Evaluation; K. Choi, Seoul National University / School of Public Health. Persistent organic pollutants (POPs) such as polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs), and polybrominated diphenyl ethers (PBDEs) have been frequently detected not only in environment but also in food and humans. Several POPs can cause adverse health effects among infants, and may also influence the health later in adulthood. Hence, levels and potential sources of major POPs and their potential adverse health effects among infants are concerns of great importance. This study was conducted to determine the levels of several POPs in maternal blood, and cord blood of fetus and mother of Korea. In addition, Health consequences of the POPs exposure, including fetal growth and hormone status are evaluated. Biological samples of 148 pregnant mother and 118 infant including 118 matching pairs were collected in five university hospital throughout the country from February to December in 2011. Questionnaire survey about general information was conducted before delivery. Target OCPs include aldrin, chlordane, HCH, DDT, endrin, toxaphene, HCB, dieldrin, heptachlor epoxide, and mirex. PCBs included congeners 28, 52, 101, 138, 153, and 180. Several PBDEs such as BDE-28, 47, 99, 100, 153, 154, 183, and 209 were also analyzed. Birth weight, birth length and head circumference for fetal growth measured, and free and total T3, T4 and TSH for hormone status were evaluated using the chemo-luminescence immunoassay and commercial kits. The association between the level of exposure to POPs and related health effects were assessed by multivariate model after adjustment for proper covariates. The sum of OCPs median concentration showed 89 ng/g lipid weight in maternal serum and 90.4 ng/g lw in cord serum. The sum of median concentration of PCB congeners 25.2 ng/g lw in maternal serum and 32.4 ng/g lw in cord serum. Among OCPs, DDT were highest measured, and among PCBs, CB 152, 138, and 52 were detected frequently. The exposure of levels of POPs among Korean pregnant mothers and matching fetuses are relatively low compared to populations of other countries. Several POPs (e.g. BDE47, PCB52, PCB153, and, pp-DDE) levels showed significant association with thyroid hormone balances and height, weight, and head circumference of newborn. The study panel will be followed up for another few years to understand the influence of prenatal POPs exposure on the development of children.

**TP194 Fish endocrine disruption responses to a major wastewater treatment facility upgrade** A. Vajda, University of Colorado Denver / Integrative Biology; L. Barber, USGS; D. Norris, J. Writer, University of Colorado Boulder. The urban-water cycle modifies natural stream hydrology, and domestic and commercial activities increase the burden of endocrine-disrupting chemicals, such as steroidal hormones and 4-nonylphenol, that can disrupt endocrine system function in aquatic organisms. This paper presents a series of integrated chemical and biological investigations into the occurrence, fate, and effects of endocrine-disrupting chemicals in the City of Boulder Colorado's WWTF and Boulder Creek, the receiving stream. Results are presented showing the effects of a full-scale upgrade of the WWTF (that treats  $0.6 \text{ m}^3 \text{ s}^{-1}$  of sewage) from a trickling filter/solids contact process to an activated sludge process on the removal of endocrine-disrupting compounds and other contaminants (including nutrients, boron, bismuth, gadolinium, and ethylenediaminetetraacetic acid) through each major treatment unit. Corresponding impacts of pre- and post-upgrade effluent chemistry on fish reproductive end points were evaluated using on-site, continuous-flow experiments, in which male fathead minnows (*Pimephales promelas*) were exposed for 28 days to upstream Boulder Creek water and WWTF effluent under controlled conditions. The upgrade of the WWTF resulted in improved removal efficiency for many endocrine-disrupting chemicals, particularly  $17\beta$ -estradiol and estrone, and fish exposed to the post-upgrade effluent indicated reduction in endocrine disruption relative to pre-upgrade conditions. Endocrine disruption responses of wild fish in Boulder Creek to the WWTF upgrade will be discussed.

**TP195 Further investigations in the environmental risk assessment of sitagliptin** J.G Tell, Merck & Company, Inc. / Global Safety & the Environment, Merck & Company, Inc. / Safety and Environment Department; L. Ziv, Merck & Co., Inc; K. Silverman, Merck & Co., Inc. / Global Safety & the Environment. Sitagliptin is the active pharmaceutical ingredient (API) of MSD's medicine for the treatment of Type II diabetes. Previous work has been presented on a prospective risk assessment for sitagliptin in the United States which included the collection of environmental fate and effects data, the assessment of future patient use of sitagliptin based on the rising prevalence of diabetes mellitus, and environmental modeling to predict future concentrations of sitagliptin in surface water and drinking water. This study has been expanded to include updated biodegradation data of sitagliptin, improved fate modeling of sitagliptin in the US and the EU. An environmental risk assessment of metformin, which is a co-API with sitagliptin, based on new environmental fate and effects data collected for this generic compound is included.

**TP196 Human Exposure and Risk Assessment for Nonylphenol and Nonylphenol Ethoxylates** T.G Osimitz, Science Strategies, LLC / President; W. Droege, Science Strategies, LLC; J.H. Driver, risksciences.net, LLC; B. Losey, Alkylphenols & Ethoxylates Research Council,. Nonylphenol (NP) and nonylphenol ethoxylates (NPE) are members of the broader chemical family of alkylphenols and their ethoxylates. NP is an intermediate used in the synthesis of other compounds, primarily NPE. NPE are surfactants with many applications including industrial and institutional and cleaning, industrial processing, the formulation of paints, adhesives and polymers and as inert ingredients in pesticide formulations. NP is found in the environment as a degradation intermediate of NPE. Our objective was to develop a risk assessment based on human exposure to NP and NPE using data available in the published literature and two complimentary approaches, one using environmental monitoring data and the other using human biomonitoring data. A critical review of all relevant publications was conducted based on standard study quality attributes. Only studies with the highest ratings were used for the assessment. First, source-specific environmental exposure data (e.g., concentrations in air, dust, food, and water) were used to derive human intake rates and exposures. These were then used to calculate source-specific Margins of Exposure (MOE) as a measure of risk associated with each exposure source. However, most of the existing environmental monitoring studies were designed to look at specific populations or locations with worst-case exposures; therefore simply adding these together would not be representative of aggregate exposure or any real population. Given the availability of human biomonitoring data for NP (e.g. concentrations in serum, urine, fat and mother's milk) calculation of exposures representative of the aggregate exposure from all sources was possible. Using pharmacokinetic data available for NP and NPE, daily absorbed dose estimates were developed for NP and MOEs were calculated

as a measure of risk for the monitored populations. The MOEs were based on the lowest No-Observed-Adverse-Effect-Level (NOAEL) for a sensitive toxicological endpoint of interest, i.e., post-natal developmental effects (13 mg/kg/day). The MOEs based on source-specific environmental exposure data ranged from 29,545 to 6,500,000. The MOEs based on the internal dose estimated from biomonitoring data ranged from approximately 2,700 to 650,000. These findings support a conclusion of reasonable certainty of no harm for human exposures to NP and NPE.

**TP197 IFRA Environmental Standards: Risk and Hazard Assessment Update for 2012** A. Lapczynski, Research Institute for Fragrance Materials, Inc., Research Institute for Fragrance Materials, Inc / Environmental Specialist; D. Salvito, Research Institute for Fragrance Materials, Inc. / Department of Environmental Science; M. Vey, International Fragrance Association. To assure safety of fragrance ingredients in consumer products, International Fragrance Association expanded the fragrance industry's self-regulatory safety program with the development of IFRA Environmental Standards for both risk and hazard in 2008. Fragrance material risk assessments for these Standards are incorporated in the Research Institute for Fragrance Materials' (RIFM) testing program in coordination with its Expert Panel. To identify materials for risk assessment refinement, fragrance materials were screened using the RIFM Environmental framework and 2008 IFRA volume of use survey as reported for both Europe and North America. The Framework for this evaluation was published in Environmental Toxicology and Chemistry (Salvito et al., 2002, 1301-1308). In addition, hazard assessment on these materials was also performed and reviewed. As a result nearly 3,000 materials were screened with preliminary risk quotients estimated to rank priority materials for risk assessment refinement. In an effort to provide greater transparency to the IFRA Environmental Standards, RIFM reports the most recent results of these additional tests (for both risk and hazard assessments) at both the annual SETAC NA and Europe meetings. These studies include persistence testing (ready biodegradation tests and die-away studies), bioaccumulation, and acute and chronic aquatic toxicity. Incorporating these new data in a second tier risk and hazard assessment for these materials will also be presented.

**TP198 Investigation into endocrin-disrupting chemicals using TG229 with Medaka** N. Tatarazako, National Institute for Environmental Studies / Center for Environmental Risk Research, National Institute for Environmental Studies / Endocrine Disrupter Research Laboratory; H. Takanobu, National Institute for Environmental Studies; H. Watanabe, National Institute for Environmental Studies / Center for Environmental Risk Research; A. Nakamura, University of Tokyo; T. Iguchi, National Institute for Physiological Sciences. "Fish Short-Term Reproduction Assay" (TG229) is the method of testing can detect adverse effects of the chemicals on reproduction adopted by OECD in 2009, such as the impact on the number of eggs, hatchability, reproductive behavior, egg quality, and spermatogenesis in addition to vitellogenin levels and secondary sexual character. It was used to detect the impact of chemicals on the hypothalamic-hypophyseal-gonadal axis and their estrogenic, antiestrogenic, and androgenic actions after 21 days of exposure. We report our results from the test performed on certain chemicals in accordance with the TG229 (17 $\alpha$ -ethinylestradiol, estrone, 4-tert-pentylphenol, tomosifen citrate, methyl testosterone, 5 $\alpha$ -dihydrotestosterone, flutamide, vinclozolin, ketoconazole and phenytoin). These chemicals except phenytoin varied in typical mode of action (MOA) relating endocrine. Phenytoin is the one of the pharmaceutical (an anticonvulsant) and was suspected to have antiestrogenic and androgenic actions by an in vitro test (reporter gene analysis). The test results do not provide a clear indication about the antiandrogenic action of these substances or their inhibitory effect on steroidogenesis, but it was confirmed that TG229 was useful for screening for substances with estrogenic, antiestrogenic, and androgenic actions. In other words the MOA of chemicals other than the antiandrogens or steroidogenesis inhibitors could be estimated from the endpoint of TG229. With phenytoin, no significant increase/decrease was observed in the secondary sexual character and the quantity of vitellogenin in the liver at any concentration. However, the total number of eggs, the number of fertilized eggs, and the fertility rate decreased significantly at higher concentration. Locomotive dysfunction including whirling, wobbling, and hyper motion was observed at all concentrations except in controls, and the degree of dysfunction increased with the concentration. Therefore, it is thought that the phenytoin had an influence on the

reproduction by behavioral disorder not endocrin-disrupting chemical. Possibility was shown assortment of the endocrin-disrupting chemicals by TG229.

**TP199 Lifetime Exposure of the Least Killifish (*Heterandria formosa*) and Western Mosquitofish (*Gambusia affinis*) to 17 $\alpha$ -Ethinylestradiol: Organism-Level Effects** L.M. Jackson, University of Louisiana at Lafayette / Department of Biology, University of Louisiana at Lafayette / Biology; P.L. Klerks, University of Louisiana at Lafayette / Department of Biology. The presence of endocrine disrupting compounds (EDCs) in the environment has gained worldwide attention because of the possibility that EDC's may be affecting the reproductive health of wildlife and humans. The synthetic estrogen 17 $\alpha$ -ethinylestradiol (EE2) is a potent endocrine modulator and is present at biologically active concentrations in aquatic ecosystems. To investigate impacts of EE2 in the aquatic environment, a full life cycle in vivo study was done to examine the potential effects of EE2 in the least killifish (*Heterandria formosa*) and western mosquitofish (*Gambusia affinis*). We tested the effects of two environmentally relevant concentrations of waterborne EE2. Newborn fishes were exposed to 1 ng/L EE2, 5 ng/L EE2, or a solvent control (ethanol) in a static-renewal system with replacement every 72 hours. Effects of EE2 on length, weight, sex ratio, survival rate, and time to sexual maturity in the developing fishes were examined. At 5 ng/L, EE2 caused a significant reduction in growth of females for both species. At 1 ng/L, EE2 exposure resulted in slower growth in female least killifish and male mosquitofish. Also, female mosquitofish exposed to 5 ng/L EE2 had a significantly lower weight at sexual maturity compared to controls. In both species at both concentrations, we noted that there was a trend of EE2-exposed males weighing more than control males – with a statistically significant difference in the mosquitofish at 1.0 ng/L. Female-biased sex ratios and decreased survival were noticed in both species at both concentrations. Finally, there was a notably longer time to sexual maturity in the EE2-exposed fishes. This ongoing study adds to the growing body of evidence of significant risks to aquatic organisms from exposure to environmentally-relevant concentrations of EE2.

**TP200 Mode of action of an antiandrogenic substance Vinclozolin using prostate cells in in-vitro cultures as a model** S. Prasad, University of Ottawa; D. Krewski, University of Ottawa / Epidemiology and Community Medicine; J.S. Gomes, Environmental Health Research Unit, University of Ottawa / Interdisciplinary School of Health Sciences, R. Samuel McLaughlin Centre for Population Health Risk Assessment, Institute of Population Health, University of Ottawa, University of Ottawa / Faculty of Health Sciences. Vinclozolin (VCZ), an antiandrogenic dicarboximide fungicide, is an endocrine disrupting chemical (EDC) commonly used on food crops. Considerable evidence suggests that VCZ and its two main metabolites, M1 and M2 possess high affinity for androgen receptor (AR) and can modulate expression of androgen dependent genes. The prostate gland is hormone sensitive and depends on AR and androgen regulated genes for proper functioning; any deviation in this process could lead to disease development. This study explores changes in viability and expression of AR, NKX3.1 and cYP3A4 genes in androgen sensitive prostate cells (LNCaP) after exposure to VCZ with and without S9 microsomes in a time and dose dependent manner. NKX3.1 is an androgen regulated gene involved in normal prostate epithelial growth and known to show aberrant expression in prostate cancer (PCa). CYP3A4 is a gene mainly involved in xenobiotic metabolism. LNCaP cells in phenol red free RPMI-1640 media were exposed to VCZ for 6, 12, 24 and 48 hours at concentrations of 100uM, 1uM and 0.01uM dissolved in DMSO. Treated and untreated cells were harvested at the end of each exposure time point. Total mRNA was extracted to determine expression of the aforementioned genes by qRT-PCR method. Cell viability was measured using MTT assay. Treatments with rat S9 liver microsomes were performed in order to examine changes in viability and expression due to vcz metabolites. VCZ showed a significant decrease in viability at higher doses after 48 hours which was slightly mitigated by treatment with S9 microsomes. Expression of NKX3.1 and cYP3A4 was upregulated to a maximum of 1.5 and 5.0 fold respectively after 24 hours. the upregulation of the androgen regulated NKX3.1 was indirectly correlated to an initial downregulation of the AR. Overexpression of cYP3A4 may metabolize VCZ but also limit the bioavailability of key endogenous steroidal hormones that are targets of CYP3A4 and also important for normal prostate function such as testosterone. S9 treated VCZ showed little change in expression of NKX3.1 and AR but only a slight upregulation of CYP3A4 and a significant



reduction in expression relative to treatment without S9 was noticed. Results from this study illustrate that VCZ can interfere with the expression of critical prostate genes including androgen regulated genes important for proper functioning of the prostate.

**TP201 Optimizing Scientific and Social Attributes of Pharmaceutical Take Back Programs to Improve Public and Environmental Health**

K.I. Stoddard, University of North Texas / Institute of Applied Sciences, University of North Texas / Institute of Applied Science; G. Maxey, C. Tiwari, University of North Texas / Department of Geography; V. Hodge, City of Denton; D.B. Huggett, University of North Texas / Department of Biological Sciences, University of North Texas / Institute of Applied Sciences. Research continues to show that pharmaceutical environmental contamination has caused adverse environmental effects, with one of the most studied effects being feminization of fish exposed to endocrine disruptors. Additionally, there are also public health risks associated with pharmaceuticals because in-home reserves of medications provide opportunities for accidental poisoning and intentional medication abuse. Pharmaceutical take back programs have been seen as a remedy to these concerns; however a thorough review of peer-reviewed literature and publicly available information on these programs indicates limited research has been conducted to validate these programs as a purported solution. Furthermore, there are significant data gaps on key factors relating to take back program participants. To address these gaps in knowledge and ultimately determine if take back programs could actually improve public and environmental health, social and scientific research was conducted on take back events called Denton Drug Disposal Days (D4). D4 events were single day events held in Denton, Texas. Socioeconomic, demographic, and geographic characteristics of D4 participants were investigated using surveys and geographic analysis. Impacts on public health were determined by comparing medications collected at D4 events with medications reported to the North Texas Poison Center as causing adverse drug exposures in Denton County. Impacts to environmental health were determined by monitoring hydrocodone concentrations in wastewater effluent released from Denton's wastewater treatment plant before and after D4 events. Data collected and analyzed from the D4 events and the wastewater monitoring program suggests D4 events were successful in contributing to improvements in public and environmental health; however there was insufficient evidence to prove that D4 events were exclusively responsible for these improvements. An additional interesting finding was that willingness to travel to participate in D4 events was limited to a five to six mile threshold. This geographic information, combined with other findings related to socioeconomic, demographic, and risk perception characteristics of D4 participants, has the potential to help improve the effectiveness of future take back events. This would allow these programs to better meet their common goals of improving both public and environmental health, which this study has shown is achievable, to some degree.

**TP202 Pharmaceuticals and personal care products on the Baca National Wildlife Refuge, Colorado, and their effects on native fish**

J.E. Zenobio, / Forestry and Natural Resources, Purdue / Forestry and Natural Resources; B.C. Sanchez, U.S. Fish and Wildlife Service / Forestry and Natural Resources; J.K. Leet, Purdue University / Forestry and Natural Resources, Purdue University; L.C. Archuleta, U.S. Fish and Wildlife Service / Forestry and Natural Resources; M.S. Sepulveda, Purdue University / Forestry and Natural Resources. The presence of pharmaceuticals and personal care products (PPCPs) in surface and ground waters has raised substantial concern, due to the difficulty to remove them by conventional drinking water treatments and their effects to aquatic organisms. These chemicals, including natural and synthetic hormones, appear in mixtures at low concentrations from areas of intense urbanization. The Baca National Wildlife Refuge, Colorado is home to several endemic fish species, including the Rio Grande chub (*Gila pandora*) and Rio Grande sucker (*Catostomus plebeius*). Within the last century, these fish species have been extirpated from much of their normal range and thus are considered vulnerable or threatened. A portion of the Refuge (South Crestone and Crestone Creeks) receives discharges of treated water from the Aspen Institute waste water treatment facility. Thus the objective of this study is to quantify PPCPs present in the sewage waste water and evaluate its potential impact to fish communities. During summer 2011, we sampled different creeks within the Refuge, including those receiving waste effluent and control creeks. During this first sampling event, we focused on fathead minnows (*Pimephales promelas*), since they are a well-studied fish model for ecotoxicological research and therefore

results are more easily interpreted. We quantified 120 analytes (antidepressants, analgesics, antibiotics, anti-inflammatory drugs, etc.) in six refuge creeks and examined their effects in fathead minnows using histological analysis of gonads and liver. Additionally, we measured the expression of six genes involved in sex differentiation and reproduction (*cyp19a*, *star*, *dmrt1*, *ar*, *era*, *vtg*). Preliminary histological analyses indicate that fish collected from South Crestone and Crestone Creeks exhibit ovarian lesions, including oocyte atresia, oocyte detachment from follicular envelope, and sinusoidal chorionic shape. Water chemistry in 2011 revealed the presence of high number of psychoactive pharmaceuticals exceeding the report limit and suggesting possible brain function alterations. Thus, we will also be studying genes involved in central nervous system function on resident fish.

**TP203 Recreating the Fish Hypothalamus-Pituitary-Gonadal Axis Feedback Mechanism in a Tissue Co-Culture System**

D.R. Johnson, U.S. Army Engineer Research and Development Center / Environmental Laboratory, U.S. Army / CEERD-EP-R, US Army Engineer Research and Development Center / Environmental Laboratory, US Army Engineer Research & Development Center / Environmental Laboratory; T. Kissane, R. Morgan, US Army Engineer Research and Development Center / Construction Engineering Research Laboratory; C. Ang, Badger Technical Services; D. Cropek, US Army Engineer Research and Development / Construction Engineering Research Laboratory; E.J. Perkins, US Army Engineering Research & Development / Environmental Laboratory, US Army ERDC / Environmental Processes & Effect Division, US Army Engineering Research & Development / Environmental Genomics and Genetics Team. Both natural and anthropogenic endocrine disrupting chemicals (EDCs) can disrupt normal hormonal production and actions within organisms, leading to dramatic physiological and reproductive dysfunctions. Whole organism studies, while the gold standard, are time and resource intensive, yet cell culture studies, which are faster, can lead to confounding and misleading data due to the lack of feedback signaling between different tissues. To address this, a tissue co-culture was created with the hypothalamus, pituitary, liver, and ovary of fathead minnow (*Pimephales promelas*). Tissues were excised from the fathead minnow, cut into small pieces, and placed in the same well of a 12-well plate. Tissue co-culture conditions were optimized with several culture media and temperatures. Tissue viability was measured after 7 days under the various culture conditions using the MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. Of the media tested, 199 Earles media retained the highest tissue viability for individuals (45% for hypothalamus, 20% for pituitary, 85% for liver, and 25% for gonads). Temperature studies demonstrated that the tissue cultures had higher viability at 19.5°C than 25°C. When tissues were co-cultured, tissue viability remained constant for the hypothalamus and pituitary, slightly decreased for liver, and increased for gonads, suggesting that the tissue co-culture provided additional factors necessary for gonadal viability. Hormonal production was also monitored over the 7-day culturing period. 11-ketotestosterone was generated at higher levels in the male HPG tissue co-culture than in the testes-only tissue culture. Finally, when challenged with fadrozole (FAD), a known EDC, the HPG axis tissue co-culture produced higher 17 $\beta$ -estradiol (E2) at 14 h than ovary tissue alone. This was demonstrated in a dose- and time-dependent manner. These results suggest that a functional HPG axis feedback mechanism can be created in vitro with fathead minnow tissues. This proof-of-concept experiment may lead to the development of more advanced tissue co-cultures that can reduce and/or replace whole animal experimentation.

**TP204 Sensitivity of Early Life-stages of Walleye (*Sander vitreus*) to 17 $\alpha$ -Ethinylestradiol**

J. Zee, University of Saskatchewan / School of Environment and Sustainability; S. Patterson, S. Beitel, J. Doering, H. Prodhall, M. Hecker, University of Saskatchewan / Toxicology Centre. Exposure to environmental estrogens has been shown to impact reproduction and development of freshwater fishes. One synthetic estrogen of concern is 17 $\alpha$ -Ethinylestradiol (EE2). EE2 is the active compound in oral contraceptive pills, and is 10-50 times more potent than natural estrogens. It enters the aquatic environment via wastewater effluents where it is commonly found in the lower ng/L range. Studies have shown that EE2 is capable of inducing feminization, and even complete sex reversal in male fish of various species at low environmentally relevant concentrations. However, little is known about the potential impacts of EE2 on fish species native to northern ecosystems. Therefore, the aim of this study was to investigate the effects of chronic exposure to EE2 on sexual differentiation and development of

walleye (*Sander vitreus*). Eyed-stage embryos of walleye were exposed to graded doses of EE2 ranging from 5 – 500 ng/L for 100 days. Subsamples were taken at 10, 30, 45, 60 and 100 days to assess impacts on different developmental stages and to identify the most sensitive life-stage. Growth and development of fish were monitored throughout the course of the experiment, and gonadosomatic (GSI) and liversomatic (LSI) indices were recorded for all fish sampled at the end of the study. At each sample time, liver and gonad tissues were collected for analysis of alterations of molecular pathways associated with sexual differentiation and development, including estrogen receptor (ER), androgen receptor (AR), vitellogenin (VTG), aromatase (CYP19), hydroxysteroid (17- $\beta$ ) dehydrogenase (hsd17b1) and zona radiata (Zr) transcript abundance. Histopathological analysis of liver and gonad samples to assess abnormalities regarding sex differentiation are underway. Overall, these analyses should establish a comprehensive understanding of the potential impacts of estrogen exposure to walleye.

**TP205 Study of the Aquatic Humic Substances action in toxicity of 17 $\alpha$ -Ethinylestradiol, 17 $\beta$ -Estradiol and Bisphenol A on the fish species *Danio rerio*** R. Fracacio, Sao Paulo State University – UNESP / Environmental Engineer; B.B. Cunha, A. Gianini, Unesp; E.N. Fragoso, N. Fenerich-Verani, UFSCar / Hidrobiologia; A.H. Rosa, Unesp / Environmental Engineering; L.C. Marais, Unesp / Environmental Engineer. The endocrine disruptors (ED) are an emerging class of contaminants present in aquatic systems worldwide. This fact justifies studies related to both its environmental performance and its role in biological systems. In addition to these understandings, it is necessary to study alternative means to minimize their potential ecotoxicological risks. In this respect, the aquatic humic substances may represent an alternative to low-cost and easy acquisition in freshwater aquatic systems. Given the above, the overall objectives of this study were to evaluate the behavior of ED Bisphenol A (BPA), 17 $\beta$ -estradiol (E2) e 17 $\alpha$ -ethinylestradiol (EE2) on test organisms adult males and females (3 months), on the fish species *Danio rerio*, in actual concentrations found in natural environments Brazilians, in the absence and presence of humic substances (SHA) extracted from river waters. The ED concentrations tested were: 1) BPA: 25 ng.L<sup>-1</sup>, 50 ng.L<sup>-1</sup> e 80 ng.L<sup>-1</sup>; 2) E2: 7 ng.L<sup>-1</sup>, 15 ng.L<sup>-1</sup> e 30 ng.L<sup>-1</sup> e 3) EE2: 6 ng.L<sup>-1</sup>, 150 ng.L<sup>-1</sup>, 300 ng.L<sup>-1</sup>. The concentration of SHA defined from preliminary tests was 20mg.L<sup>-1</sup>, extracted from the river Itapanhaú (southeastern Brazil). These experiments lasted 15 days and followed the semi-static regime, with a total exchange of test solution every 3 days. The results showed that the males fish exposed to 300 ng L<sup>-1</sup> of EE2 it was observed atrophy of the hepatocytes of the liver. However, in the presence of 20 mg.L<sup>-1</sup> of SHA, histological changes were not identified. The other ED showed no significant biological effects and no histological changes of liver and gonads in the concentrations and conditions studied.

**TP206 Testicular Estrogen Production in a Flatfish from Southern California Waters: Environment Associated Differences, Underlying Molecular Mechanisms** C. Larsen, California State University, Long Beach / Biology department; J. Reyes, Pacific Coast Environmental Conservancy; E. Iwanski, California State University, Long Beach; K. Kelley, California State University, Long Beach / Environmental Endocrinology Laboratory, California State Univ, Long Beach. In selected locations offshore of urban southern California, males of the marine fish, *Pleuronichthys verticalis* (hornyhead turbot), have been found to have elevated plasma concentrations of the female steroid, 17 $\beta$ -estradiol (E2). Over several years of study, it has been observed that males sampled from within Santa Monica Bay (offshore of Los Angeles) have consistently elevated E2 levels (often >1000 pg/ml), as much as 10 times higher than in males sampled from down-coast locations offshore of Orange County. Since estrogens including E2 are at undetectable levels in the ocean discharge areas of the regional wastewater treatment plants (WWTPs), it was of interest to determine whether testicular expression of steroidogenic enzymes involved in estrogen production may be altered (by a putative environmental endocrine disruptor?) and linked to endogenous E2 production. Results indicate that gonadal expression of StAR, P450aromA and 17 $\beta$ -HSD1 measured by qPCR were all relatively higher in testis of fish from Santa Monica Bay, and levels of expression were significantly correlated with E2 concentrations. Furthermore, rearing of fish caught from Santa Monica Bay (with high E2 levels) in aquaria containing clean seawater for 4 and 8 weeks resulted in subsidence of the high estrogen phenotype and testicular expression of the steroidogenic genes, down to levels seen in turbot and other flatfish males from outside of the region (< 100 pg/ml). Using proteomics approaches, screening of protein expression

in testis from high E2 males is leading to additional candidate mechanisms underlying the apparent environment-associated increases in testicular E2 production in turbot. (Supported by NOAA/USC Sea Grant Program)

**TP207 Testicular Oocytes in Largemouth Bass (*Micropterus salmoides*) on the Delmarva Peninsula** L.T. Yonkos, University of Maryland, Wye Research and Education Center / Wye Research & Education Center, University of Maryland / Wye Research & Education Center; E.A. Friedel, University of Maryland / Wye Research and Education Center; D.J. Fisher, University of Maryland / Wye Research & Education Center. Reports of intersex fish in US waters have become a common occurrence over the past decade. National and Mid-Atlantic regional surveys of freshwater fish species suggest both largemouth (LMB; *Micropterus salmoides*) and smallmouth bass (SMB; *Micropterus dolomieu*) are uniquely sensitive to gonadal feminization in response to exogenous estrogens. We describe the prevalence and severity of testicular oocytes (TO) in LMB collected over a five year period (2005 – 2009) from a variety of surface waters (e.g., small order streams, ponds, lakes and rivers) on the Delmarva Peninsula. Several sites were sampled pre- and post-spawn and/or multi-year to investigate seasonal and annual variations. During 2005-2007 common fish and frog species were collected via backpack electroshocker from approximately 20 small-order streams within four major Delmarva river systems. Approximately 500 specimens representing 12 fish and three frog species were collected and gonads examined histologically. Of all species collected, only LMB had evidence of TO (SMB are not present on Delmarva). Severity was minimal and prevalence difficult to determine because few LMB were present at individual stream sites. Subsequent fish were collected from larger water-bodies (via boat electrofishing) to ensure sufficient numbers of LMB to assess prevalence and calculate severity indices (SI). During 2008, approximately ten male LMB were collected from each of six Delmarva lakes with collection activity occurring during late spring and early summer. Intersex was encountered in fish from all six lakes (100%); prevalence within sites ranged from 40% to 88%; mean across sites was 65%. In 2009 male LMB collected seasonally from a previously sampled lake and the main channel of a Delmarva river had TO prevalences of 50% (spring) to 33% (summer) and 33% (spring) to 80% (summer), respectively. Collectively the prevalence of TO in Delmarva LMB collected in 2008 and 2009 (range 33% to 88%) was comparable to levels reported nationally for LMB from impacted surface waters and for SMB from Mid-Atlantic regional waters. Site average SI ranged from 0.11 to 0.46 (unitless measure), generally lower than reported SI in SMB collected from impacted reaches of the Potomac River system (e.g.,  $\geq 0.60$ ), but higher than those reported for SMB from minimally impacted reference sites outside of the Potomac Drainage (e.g.,  $\leq 0.10$ ).

**TP208 The Effects of Three Phytoestrogens Single and in Mixture on Anatomy, Physiology and Behavior of Fathead Minnows** D.C. Rea- ick, St. Cloud State University; N. Fleischacker, P. Novak, University of Minnesota; H. Schoenfuss, St. Cloud State University / Aquatic Toxicology Laboratory, St. Cloud State University / Department of Biological Sciences WSB-273, St. Cloud State University / Department of Biological Sciences MS-273. The contributions to total biological activity within aquatic environments by phytoestrogens from natural sources and anthropogenic effluents is poorly understood. While lower in estrogenicity compared to other endocrine active compounds such as estradiol and ethinylestradiol, phytoestrogens have been linked to organismal response below pulp mill effluent. As a broad class of compounds with an unknown distribution, phytoestrogens have potential to influence individuals through development and behavior. In replicate flow-through exposure experiments, we assessed the anatomical (secondary sex characteristics, histology), physiological (vitellogenin induction) and behavioral (nest defense) consequences of fathead minnow exposure to daidzein, genistein and formononetin single and in mixture at environmentally relevant (333 and 1,000 ng/L each) concentrations. Groups of 20 male and 20 female fathead minnows were exposed for 21 days under controlled laboratory conditions. Following exposure, half of the fish were sacrificed for anatomical and physiological endpoints while the remaining fish were placed as breeding pairs (within each treatment) in separate spawning aquaria. Nest holding behavior and reproductive output was monitored daily for 10 days. We also assessed the effects of biological breakdown products of these three phytoestrogens in a follow-up experiment. Anatomical endpoints, including secondary sex characteristics and testis tissues were unaffected by the exposures. Daidzein and the phytoestrogen mixture were slightly (but statistically significant) estrogenic as indicated

by plasma vitellogenin concentrations in exposed male fathead minnows. Nest defense behavior by male fathead minnows was not altered by any of the treatments. Funding provided by the MN LCCMR.

**TP209 Toxicity Evolution Along Oxidative Degradation of EDCs** N. GOU, Northeastern University / Civil & Environmental Engineering, Northeastern University / Civil and Environmental Engineering; S. Yuan, A.N. Alshawabkeh, Northeastern University; A. GU, Northeastern University / Department of Civil & Env Engineering, Biotechnology Initiative Program, Department of Civil & Env Engineering / Biotechnology Initiative Program. Endocrine Disrupting Compounds (EDCs) exhibit severe health impact on aquatic organisms and human. Removal of EDCs from water sources is required to eliminate the associated health risks. Various treatment technologies have been explored to remove EDCs from water and advanced oxidation process has been shown to be most promising. Yuan et al recently demonstrated the application of a novel electro-fenton process for degradation of organic contaminants [1]. Various intermediates have been reported to occur during the oxidative degradation process, however, toxicity along the degradation process have never been evaluated. This is mostly due to the lack to applicable toxicity assessment methods. In this study, we applied a novel toxicogenomics-based toxicity assessment methods using whole cell array to monitor the toxicity changes and their association with intermediates produced during the oxidative degradation of 3 EDCs via electro-fenton reactions. For each single compound, more than 70-80% of the parent compounds disappeared after 15mins treatment, however, toxicity level did not decrease much. For bisphenol A and ibuprofen, there seemed to be no reduction in the overall toxicity at 15min, indicating persistent or increased toxicity of the intermediate products generated. For triclosan, toxicity was significantly reduced as the parent compound transformed, likely with dechlorination step at the beginning. Extended oxidation process for 30-45 mins led to further degradation of intermediates and corresponding reduction of toxicity as well. We then evaluated the toxicity changes during the degradation of the mixture of the above chemicals in both DI water and wastewater matrix. Samples with mixture of the 3 chemicals showed relatively higher toxicity level than each individual compound. Interestingly, chemical disappear rates and toxicity reduction rates in the sample with wastewater matrix were higher than those in DI water despite the fact that the higher initial toxicity of the former. Our results for the first time demonstrated that the toxicogenomic approach could be potentially applied as a feasible method for quantitative water toxicity assessment. The sensitivity and specificity are sufficient to distinguish among different chemicals and different background matrix. The results of toxicity evolutions along contaminants degradation process have important implications related to treatment process design and optimization.

**TP210 Effects of Non-Selective Cyclooxygenase Inhibitor, Ibuprofen, on Gene Expression, Prostaglandin Synthesis and Reproductive Behavior in Zebrafish** T. Firkus, University of Saint Thomas, University of St. Thomas; J. Marchuk, University of Saint Thomas / Dept. of Biology; D. Martinovic, University of Saint Thomas, University of St. Thomas / Dept. of Biology, U.S. EPA, Mid-Continent Ecology Division / Mid-Continent Ecology Division, University of St. Thomas / Biology, University of Saint Thomas / Biology. Prostaglandins are a class of hormones important for the regulation of steroidogenesis and ovulation in vertebrates. In fish they are also used as pheromones; they are released by ovulating females and initiate and synchronize male reproductive behaviors. Pharmaceuticals such as nonsteroidal anti-inflammatory pharmaceuticals (e. g. ibuprofen (IB)) inhibit cyclooxygenase (COX) enzymes which catalyze prostaglandin synthesis. Sexually mature zebrafish males (n=14) and females (n=14) were exposed to 50 µg/L of IB for 14 days and their reproductive behaviors were compared to well-water exposed controls (n=14 per sex). The present study also examined effects of IB exposure on prostaglandin synthesis pathway; we measured phospholipase, COX 1 and 2, and prostaglandin synthase mRNA abundance using real time polymerase chain reaction. Also, the ovarian prostaglandin F2 alpha concentrations and COX enzyme activity were evaluated. Exposure to IB caused decrease in COX activity and was mirrored downstream in the reduced production of PGF-2alpha. Our lack of ability to detect effects on PGF-2alpha was likely a result of a high variability due to the asynchronous ovarian maturation. Furthermore, exposed fish may have been able to compensate for the decreased COX activity by increasing abundance of mRNA for COX and prostaglandin synthases (as demonstrated by our gene expression data). Several courtship behaviors were significantly

decreased in IB-exposed males, but they were not altered in females. These findings suggest that IB affects prostaglandin synthesis pathway and impairs performance of male sexual behavior and thus has a potential to impair the reproductive success of exposed individuals.

**TP211 Endocrine Disrupting Compounds in Wyoming Surface Waters: Assessment of Presence and Suspected Sources** E.O. Johnson, H.L. Bergman, University of Wyoming / Department of Zoology & Physiology. In the last two decades, studies in North America and Europe have demonstrated the widespread occurrence of intersex condition and other reproductive abnormalities in fish related to the presence of endocrine disrupting compounds (EDCs) in surface waters. Many of these studies point to the discharge of steroid hormones and industrial chemicals from wastewater treatment plants (WWTPs) as the principle source of these abnormalities, but other sources of EDCs have been identified such as the deposition of atmospheric contaminants and leaching of steroid hormones from livestock manure. Because there is little information about the presence of EDCs in Wyoming, and because livestock outnumber the human population more than 2 to 1, Wyoming provides a unique scenario for studying the impacts of EDCs from both WWTP and agricultural sources. Pursuant to assessing the presence and sources of EDCs in Wyoming, we collected white suckers (*Catostomus commersonii*) in the fall of 2011 and the spring of 2012 from three sites on the Laramie River including (1) a site upstream of potential runoff from Wyoming rangelands, (2) a site downstream of potential runoff from Wyoming rangelands, and (3) a site immediately downstream of the Laramie WWTP discharge. Histological examination of the gonads revealed increased intersex condition, increased oocyte atresia and female dominated sex ratios in downstream fish, and enzyme-linked immunosorbent assays showed elevated plasma vitellogenin concentrations. Water samples were taken from all three sites concurrent with the spring fish collection and will be analyzed for known EDCs including: steroid hormones, alkylphenols and perfluorinated compounds. The second phase of the project will include the 7-day deployment of caged fathead minnows (*Pimephales promelas*) during the summer of 2012 in the Laramie River and at additional sites throughout Wyoming, followed by quantification of vitellogenin transcripts by qPCR. The results of this statewide survey will also be presented.

**TP212 Alternatives for Derivation of Aquatic Life Criteria for Copper using the Biotic Ligand Model** J. Gondek, GEI Consultants; B.W. Gensemer, GEI Consultants / Ecological Division; S.D. Baker, GEI Consultants, Inc. / Ecotoxicologist/Wildlife Biologist; C. Claytor, GEI Consultants, Inc.; J. Gorsuch, Copper Development Association. The Biotic Ligand Model (BLM) is currently the most scientifically advanced method for deriving federal site-specific ambient water quality criteria for copper. The EPA has encouraged the use of BLM-based copper water quality criteria and several states are reviewing or beginning to adopt the use of BLM-based methods for derivation of copper criterion on a site-specific or statewide basis. While many states and agencies will be transitioning from the outdated hardness-based criteria, there is little standardized guidance for the implementation of BLM-based site-specific copper criteria. Here we explore three potential methods for deriving criteria that incorporate information from BLM: instantaneous criteria derived during most sensitive water quality conditions, BLM-derived Water-Effects Ratio (WER), and the Fixed Monitoring Benchmark. Using water quality data from Colorado river basins, these three methods were compared for their level of protectiveness under varying geochemical and temporal conditions. In particular, instantaneous criteria were often the most restrictive, and tended to be overly protective for most other times of the year, while the high hardness characteristic of Colorado waters resulted in much higher WER-based criteria compared to other methods. These results suggest that the three methods offer considerably different criteria values which raise the question of "which would be the most scientifically defensible or most practical to implement?" This study aims to simplify the implementation of the BLM for copper criteria and offer recommendations on the benefits, drawbacks and most practical uses of these methods from a scientific and regulatory perspective.

**TP213 Assessment of mercury and organic matter in thermokarst affected lakes of the Mackenzie Delta uplands, NT, Canada** R. Deison, Ottawa University / Biology; J. Blais, Ottawa University. Total permafrost in the Northern Hemisphere currently occupies an area of 26 million km<sup>2</sup>, and by 2100, this area is expected to decrease by 19-35%. In the Mackenzie Delta, NWT, temperatures are projected to rise by 4 to 5°C in the next 50



years. Over the past 20 years, mercury and PCBs have been steadily rising in burbot from the Mackenzie River, which has prompted speculation on how the changing physical environment, such as thawing permafrost, might be affecting contaminant cycles in these thermokarst environments. We examined sediments from 14 thermokarst affected lakes in a paired design where 7 lakes had retrogressive thaw slumps on their shorelines and 7 lakes were reference lakes without thaw slumps. We showed thaw-affected lakes had higher sedimentation rates and lower total Hg, methyl mercury (MeHg), and labile OC fractions compared to reference lakes. Total Hg and MeHg concentrations were correlated with total organic carbon (TOC), S2 (labile algal-derived OC), and sediment inferred chlorophyll *a* content, indicating an association between autochthonous organic carbon and Hg in these sediments. Dissolved organic carbon, total phosphorus, soluble reactive phosphorus, and total and methyl mercury were significantly lower in lakes with retrogressive thaw slumps than reference lakes, likely due to deeper water infiltration through clay-rich tundra soils. These results provide compelling evidence that thaw slumps near lakes of the Mackenzie Delta uplands are not responsible for the rising trend in mercury concentrations of fish in the Mackenzie River.

**TP214 Copper Toxicity and Acclimation to Copper using Behavioral Endpoints of Shoaling in the Least Killifish (*Heterandria formosa*)** S.K. Vogt, Arkansas State University / Biology; A. Billock, UNIVERSITY OF LOUISIANA AT LAFAYETTE / Biology; P.L. Klerks, UNIVERSITY OF LOUISIANA AT LAFAYETTE / Department of Biology. Copper's effects on organism survival and at the sub-organismal level have been extensively studied. Due to copper's known neurotoxic effects, I wished to determine whether shoaling behavioral response could be used as indicators of acute copper toxicity and endpoints in studying acclimation in least killifish (*Heterandria formosa*). For acute toxicity, least killifish were exposed to 0, 25, 50, or 100 µg/L Cu for a 2-hour duration and then tested for changes in shoaling behavior. The occurrence of copper-acclimation was tested by pre-exposing fish to either 0 µg/L or 15 µg/L Cu for a 7-day duration, then exposing them to 25, 50, or 100 µg/L Cu to assess whether the two pre-exposure groups differed in their behavioral responses to high Cu levels. Behavioral responses were tested in a subdivided aquarium, with one side containing a group of conspecific fish and the other side the focal fish whose behavior was recorded for three minutes. Shoaling was based on the proximity of the focal fish to the divider separating it from the group of fish (in shoaling experiments) or the predatory fish (in predator avoidance experiments). The occurrence of shoaling was confirmed for this study's design. Acute copper exposure had a significant effect on both time-to-first shoaling and total amount spent shoaling. Experiments assessing the occurrence of copper-acclimation using shoaling as the toxicity endpoint did not detect a difference between the pre-exposure groups for the effect of copper on the shoaling variables. Overall, this study demonstrated that acute copper exposure can alter shoaling behavior. However, shoaling behavior appears to be relatively insensitive as an endpoint for assessing copper acclimation.

**TP215 *Daphnia magna* immobilization assay application to toxicity of metal ions; Cesium** S. Takahashi, T. Sugawara, Tsuruoka National College of Technology / Dept. of Chemical and Biological Engineering; T. Abe, Tsuruoka National College of Technology / Dept. of Chemical and Biological Engineering, Tsuruoka Nat. College of Technology / Dept. of Material Engineering. Various metal ions exist in environment, their come from not only natural source but also artificial source. Recently the diffusions of cesium, strontium and iodine induced radioactive environmental pollution because of the Fukushima Daiichi Nuclear Power Station accident in the Great East Japan Earthquake which occurred on March 11, 2011. The cesium-137 which is an isotope of cesium has radioactivity, and generates it by the nuclear fission of uranium-235. Of course radioactive compounds and element (radioisotope: cesium-137, strontium-90, iodine-131) affect organisms and disrupt ecosystems. We expect that the not-radioactive metal ion also affect organisms (stable isotope: cesium-133, strontium-88, iodine-127). Since metal chloride is one of the stable forms, the toxicity of metal chloride is investigated with *Daphnia magna* immobilization assay. Cesium chloride (CsCl), potassium chloride (KCl), and rubidium chloride (RbCl) are used as test reagent. *D. magna* immobilization test was carried out based on OECD Guidelines for the Testing of Chemicals (TG202). 20 neonates within after the birth 24 hours exposed with metal ions under static condition at constant temperature 20±2 degree C. The neonates were observed at 24 and 48 hours after exposure and recorded the number of

survived neonates. The EC<sub>50</sub> of CsCl was 67.0 mg/L, KCl was 800 mg/L, and RbCl was 87.0 mg/L. These results showed that it was valuable to evaluate inorganic compound toxicity with *Daphnia magna*.

**TP216 Effect of copper on mitochondrial respiration thermal performance curves in rainbow trout, *Oncorhynchus mykiss*** M.M. MacDougald, Atlantic Veterinary College / Biomedical Science; C. Kamunde, University of Prince Edward Island / Associate Professor, University of Prince Edward Island / Assistant Professor. Because of the highly significant effect of temperature on animal physiology, assessing how climate change influences fish responses to pollutants is an important aspect of environmental toxicology. Among the responses at the lower levels of biological organization, those on cellular energy homeostasis are highly informative because they can be correlated with environmentally relevant assessment endpoints including mortality and growth and reproductive impairment. Here we investigate the effect of temperature on mitochondrial respiration thermal performance curves *in vitro* as a foundation for comprehensive *in vivo* and field studies. Mitochondria were isolated from rainbow trout (140-180g) livers by differential centrifugation and were, in the first set of experiments, exposed to Cu (0.01-1 mM) at 5, 11, and 25 °C during active oxidative phosphorylation supported by succinate, a mitochondrial enzyme complex II (mtCII) substrate. In the second set of experiments, un-energized mitochondria were incubated for 1 hour with lower concentrations (0.01-10 µM) of Cu to measure the effects on mtCII activity. We observed increased oxygen consumption rates and enhanced sensitivity of mitochondrial respiration to Cu at higher temperatures. Copper exposure impaired oxidative phosphorylation by stimulating proton leak and by inhibiting state 3 respiration. Additionally, mtCII activity was reduced by Cu exposure indicating that direct effects on this enzyme contribute to the impaired mitochondrial function. Overall, while our data suggest that mitochondrial endpoints of toxicity may be useful for assessing and monitoring effects of climate change on Cu (metals) toxicity in fish, studies at higher levels of biological organization are necessary to substantiate this assertion.

**TP217 Elevated temperature sensitizes rainbow trout mitochondria to copper toxicity** R. Sappal, University of Prince Edward Island; C. Kamunde, University of Prince Edward Island / Associate Professor, University of Prince Edward Island / Assistant Professor. Understanding the confounding effect of temperature fluctuations on metals toxicity in aquatic systems has experienced renewed interest due to the global climate change phenomenon. We investigated the effects of temperature stress on copper (Cu)-induced mitochondrial toxicity *in vitro* to reveal the mechanisms of temperature-Cu interactions on mitochondrial bioenergetics. Mitochondria were isolated from rainbow trout (80-130g) livers by differential centrifugation and exposed to Cu (0.01-2.5 mM) at 5, 11, 15, and 25 °C. States 3 and 4, and uncoupler-stimulated (UCS) mitochondrial respiration rates were measured using Clark-type oxygen electrodes. Copper exposure inhibited UCS respiration indicating impairment of the electron transport chain. Because the UCS respiration inhibition correlated with reduced malate/glutamate-driven respiration, we concluded that mitochondrial complex I is impaired by Cu. The Cu dose-response relationship depended on the state of respiration. Whereas state 3 respiration was monotonically inhibited by Cu exposure, low and high Cu concentrations stimulated and inhibited state 4 respiration (proton leak), respectively. Moreover, high temperature increased mitochondrial respiration rates and enhanced Cu mitotoxicity. The Q<sub>10</sub> effects for state 3 and 4 respirations were higher at lower temperatures and the thermal dependence of oxidative phosphorylation was lost at high Cu concentrations. Taken together, Cu impairs oxidative phosphorylation and stimulates proton leak in rainbow trout mitochondria, with high temperature exacerbating these effects. Thus elevated temperature, as envisaged to occur due to global climate change, may sensitize fish to metals toxicity through increased impairment of cellular energy metabolism.

**TP218 Experiments Measuring Bioavailability in Oxic and Spiked Sediments** A. Harrison, D. Costello, University of Michigan / School of Natural Resources & Environment; A. Burton, University of Michigan / School of Natural Resources & Environment and Cooperative Institute for Limnology & Ecosystem Research; C. Hammerschmidt, Wright State University / Department of Earth & Environmental Sciences; L. Podzikowski, R. Mendonca, University of Michigan. Sediment metals may be toxic to aquatic biota, but toxicity is highly dependent on bioavailability. Metal partitioning and bioavailability in sediments has been primarily linked to

sulfides and organic carbon, yet metals can also sorb to Fe- and Mn- oxides in aerobic sediments. Our research aims to improve metal bioavailability models for stream ecosystems by incorporating an oxic partitioning component. Specifically, the development of an empirical model that includes binding by Fe- and Mn-oxides, which appear to be important in binding of metals under oxic conditions. Two reference sediments, with differing binding capacities, were spiked with five concentrations each of Cu and aged under flow-through conditions in the lab while concurrently exposing *Hyalella azteca* to those sediments to measure changes in toxicity as the sediment ages. Frequent temporal sampling produced a fine scale understanding of geochemical and toxicological dynamics in the sediment, and ultimately determined the length of time necessary for lab-spiked sediments to reach a steady-state in an oxic environment. Metal release from spiked sediments rapidly decreased through time when exposed to flowing waters. As predicted, toxicity declined during the equilibration as Cu was absorbed to Fe oxides in sediments and made less available for uptake by *H. azteca*. The flume aged sediments can be used as surrogates for field contaminated stream sediments, allowing for more accurate predictions of effects under natural conditions. The sediment aging studies will also be useful for creating lab-to-field correction factors for sediment spiking.

**TP219 Mercury in the Grand Bay National Estuarine Research Reserve: An Initial Reconnaissance in Preparation for Long-Term Studies** C. Jagoe, Florida A&M University / Environmental Sciences Institute, Florida A & M University / NOAA Environ Coop Science Center/ Environ Sciences Inst; W. Luke, NOAA / Air Resources Laboratory; C. Mohrman, NOAA Environmental Cooperative Science Center, Florida A&M University; M. Woodrey, Mississippi State University / Coastal Research and Extension Center. Grand Bay National Estuarine Research Reserve (GBNERR) in coastal Mississippi is ideally suited for studies of mercury (Hg) in Gulf coastal environments, because it has a NOAA-ARL monitoring station that is a member of the National Atmospheric Deposition Program's (NADP) Atmospheric Mercury Network (AMN) and also has ongoing ecological research programs to support ecotoxicology studies. Gaseous and particulate mercury concentrations have been measured continuously since 2006-2007, and wet deposition since 2010. Total Hg deposition in the area is approximately 20-30  $\mu\text{g M}^{-2} \text{yr}^{-1}$ . To assess spatial gradients across the reserve, sediment was collected from 60 stations in GBNERR in March and July 2010. Total Hg did not differ between seasons, and ranged from 0.2 to 69.6 (median = 18.1)  $\text{ng g}^{-1}$  (ppb) dry weight. Sediment Hg differed among transects, and among locations within transects, and was positively (but weakly) related to organic matter ( $r^2=0.25$ ). About 40% of the total variation in Hg occurred within aliquots from the same grab sample, suggesting that small-scale variations in sediment composition are a major factor influencing sediment Hg accumulation. For fish, Hg was lowest in mullet (median 12  $\text{ng g}^{-1}$ ) and higher in hardhead catfish, croaker and pinfish (medians 189, 196 and 292  $\text{ng g}^{-1}$  dry weight, respectively); none exceeded EPA risk-based consumption guidelines. Oyster and shrimp Hg was 100-150  $\text{ng g}^{-1}$  dry weight, but concentrations in snails were considerably higher (median 460  $\text{ng g}^{-1}$  dry weight). Measurements of total and methyl Hg in water at multiple stations are underway. Mercury concentrations in Grand Bay sediments and biota are relatively low, reflecting a relatively pristine area receiving Hg via atmospheric deposition and runoff. Future studies will address spatial and temporal variation in mercury accumulation and speciation. We plan a long term, multidisciplinary effort to construct Hg budgets, assess Hg input, methylation, accumulation and possible biological effects, and detect any temporal trends in the reserve.

**TP220 SELDI-TOF MS approach to evaluation of heavy metals toxicity of field collected samples on the *Heterocypris incongruens*** Y. Kim, Korea University / Division of Environmental Science and Ecological Engineering, College of Life and Environmental Sciences; H. Mo, Korea University / Division of Environmental Science and Ecological Engineering; Y. Lee, Korea University / Division of Environmental Science and Ecological Engineering, College of Life and Environmental Sciences, Korea University / Environmental Science and Ecological Engineering; M. Kim, Korea University; S. Yu, Korea Univ.; K. Cho, Korea University / Division of Environmental Science and Ecological Engineering, College of Life and Environmental Sciences, Korea University / # 407, Division of Environmental. The overall objective of this study was to develop the evaluation method of heavy metals toxicity on the *Heterocypris incongruens*. Three stage experiments were performed: Establishment of the SELDI-TOF analysis condition

using the *H. incongruens*, protein expression profiling of the test animal under heavy metal exposure, and application of the evaluation method to the field sample. Q10 protein chips, Tris-HCl buffer and saturated sinapinic acid matrix (SPA) were selected for the best analysis condition. Protein expression profiling of the *H. incongruens* was conducted for the three different heavy metals, cadmium, copper and lead under selected analysis condition. Obvious differences in the peak patterns (up- and down- expression of protein in *H. incongruens*) between the heavy metals were observed, and then twenty peaks that significantly differed between the exposure conditions were selected for the evaluation of the field sample. Data analysis by non-metric multi-dimensional scaling (MDS) was performed for the evaluation of the field samples. MDS plots revealed good separation of groups between polluted site and reference site samples. Overall, the results show the potential of SELDI-TOF MS as an evaluation tool for comparing responses under the field condition, and for evaluating exposure as well as toxicant specific responses.

**TP221 Sex and habitat-specific foraging drive mercury concentrations in benthic and limnetic ecotypes of stickleback in Benka Lake, Alaska** J.J. Willacker, F.A. von Hippel, University of Alaska Anchorage / Department of Biological Sciences; K.A. Ackerly, Adelphi University / Department of Biology; T. O'Hara, University of Alaska Fairbanks / Institute of Arctic Biology. Mercury (Hg) is a widespread environmental contaminant known for the neurotoxicity of its methylated forms which bioaccumulate and biomagnify in aquatic food webs. Mercury bioaccumulation and biomagnification rates are known to vary among species utilizing different food webs (benthic versus limnetic) within and between systems. We used stable isotopes of carbon and nitrogen to determine if differences in total Hg (THg) concentration and biomagnification exist between sympatric benthic and limnetic ecotypes of threespine stickleback fish (*Gasterosteus aculeatus*) from Benka Lake, Alaska (USA). The average THg concentration in the limnetic ecotype was significantly higher than that of the benthic ecotype. Trophic position and percent benthic carbon utilized were both important determinants of THg concentration; however, in females the two variables were of approximately equal importance while in males trophic position clearly explained more of the variance than percent benthic carbon. Additionally, strong sex effects were observed in both ecotypes with female fish having lower THg concentrations than males. These results indicate that trophic ecology and sex are both important determinants of Hg contamination even within a single species and lake, and likely have important implications for Hg concentrations in higher trophic levels.

**TP222 Speciation of heavy metals/metalloids in electronic waste recycling site using X-ray absorption fine structure spectroscopy** T. Itai, Ehime University / Center for Marine Environmental Studies (CMES); S. Takahashi, K.A. Asante, Ehime University; M. Otsuka, Ehime University / Center for Marine Environmental Studies; T. Agusa, Center for Marine Environmental Studies (CMES) / Shimane University, Center for Marine Environmental Studies (CMES), Ehime University / Ehime University, Ehime University / Ehime University, Ehime University / Center for Marine Environmental Studies (CMES); A. Subramanian, Ehime University; S. Tanabe, Ehime University / Center for Marine Environmental Studies. Rapidly developing Asian and African countries face an increasing amount of e-waste, both, from domestic generation and illegal imports. For emerging economies, these material flows from waste imports not only offer a business opportunity, but also satisfy the demand for cheap second-hand electrical and electronic equipment. Uncontrolled procedure of e-waste recycling can induce environmental pollution, since e-waste contains more than 1000 different substances, many of which are toxic, such as lead, mercury, arsenic, cadmium, selenium, hexavalent chromium, and flame retardants that create dioxins emissions when burned. Since workers in recycling area are likely exposed to the various toxic materials, risk assessment criteria will be complicated. Although speciation of metals/metalloids in soil from e-waste recycling area is important to assess toxicity and mobility of them, this information is poorly reported. While primary chemical form of metals can be estimated, change in chemical form possibly occurs during recycling process. In this study, we chose two e-waste recycling sites in Accra (Ghana) and Bangalore (India). Soil and ash mixtures were collected from open burning site where some electric products were burned to recover Cu. Speciation of Cl, Cu, Zn, As, Br, Pb in soil/ash mixtures were conducted using X-ray absorption fine structure spectroscopy. The results indicated that Copper and Zn showed some variation and predominant species were likely  $\text{CuCl}_2$



and ZnS, respectively. Since Cu is originally metallic form, it could be converted to CuCl<sub>2</sub>, which can catalyze dioxin formation, during open burning process. Predominant As species were arsenate which is less toxic form than As<sub>2</sub>O<sub>3</sub>, most common As compounds in industrial usage. This result also reflected possible conversion during open burning. Majority of Cl and Br were inorganic salt despite original forms were likely organic compounds such as flame retardants. Predominance of inorganic Br in soil/ash mixtures might be attributed to the conversion of organic Br by thermal decomposition. Considering the all result, formation of dioxin like compounds is probably high in this area and hence further comprehensive monitoring is needed to assess the health risk for the e-waste recycling workers.

**TP223 Stress (cadmium) response in *Daphnia magna*: A model organism for ecotoxicology** S. Roy, University of Notre Dame / Biology; M. Pfrender, University of Notre Dame. Environmental pollution due to industrialization and urbanization is a major concern for environmental scientists. Industrial effluence is a major sources of contamination to freshwaters increasing the risk of exposure for aquatic organisms.. One ubiquitous pollutant is heavy metals that pose a danger not only to aquatic organisms, but also to human health through drinking water. To develop risk assessment models and water quality criteria environmental protection agencies (e.g., U.S. Environmental Protection Agency (US EPA)) throughout the world use laboratory toxicity assessments. While informative, these acute tests are limited to gauging organismal tolerance to heavy metals or other contaminants, and are often restricted to a handful of model organisms. These assessments do not predict the consequences of chronic low-dose exposures (a real world situation) at the individual or population level. Our research addresses such questions using freshwater aquatic invertebrate *Daphnia magna*. *Daphnia* has long been studied as a model organism in ecology, evolutionary biology and toxicology research. Our goal is to investigate adaptive mechanism of *Daphnia magna* while exposed to cadmium as one of the major contaminant present in various water-bodies in North America. Our investigation indicates that *Daphnia* from different geographic regions (with no cadmium environment) showing different level of cadmium response.

**TP224 Survival, malformation, and swim-up success of rainbow trout, brook trout, and cutthroat trout larvae from Se-fed females** N. Pilgrim, J. Rasmussen, University of Lethbridge; V.P. Palace, Department of Fisheries & Oceans Canada, Stantec Consultants; L. Ripley, Alberta Sustainable Resource Development; A. Hontela, University of Lethbridge / Dept. of Biological Sciences. Selenium (Se), an essential element, toxic at concentrations only slightly higher than those required for homeostasis, is converted to seleno-methionine (SeMet) in the aquatic environment and can accumulate in fish tissue causing detrimental effects. Selenium is transferred from the female to developing embryos where it may cause malformations. The toxicity of Se varies among closely related species; with rainbow trout ranked as more sensitive than brook trout. This study examined the effect of Se on larvae from adult rainbow trout, brook trout, and cutthroat trout fed Se-methionine enriched diets for 5 months. Selenium was measured in adult tissues, larvae and the diet. A vertical incubator was used to estimate larval survival and malformations, and a specially designed gravel-bed flume system was used to estimate the swim-up success of the larvae. A significant negative correlation between survival and egg Se was observed in rainbow trout and cutthroat trout larvae in each developmental stage, while brook trout had decreased survival in the first stage only, spawned to eyed eggs. Rainbow trout and cutthroat trout larvae also had decreased swim-up success with increased egg Se, but no effect of Se was detected in brook trout. Malformations of the larvae were evaluated for the three fish species, however the only significant trend detected was an increase in edema for cutthroat trout with increasing egg Se. The results of this study suggest that survival of larvae and swim up success rather than malformations, are the endpoints highly correlated to Se burdens and fish species. This study provides new information about Se toxicity in larvae of three salmonid species studied under a controlled laboratory setting. (Funded by NSERC MITHE-SN and Alberta Conservation Association).

**TP225 Use of field collected aquatic macroinvertebrates to determine acute zinc toxicity** S.B Skigen, S.P. Canton, GEI Consultants, Inc. / Ecological Division. Toxicity of zinc to typically sensitive aquatic macroinvertebrates (mayflies, stoneflies, and caddisflies or "EPT taxa") in Colorado streams is relatively unknown and of particular interest when considering

applicable zinc standards for coldwater trout streams. The lack of laboratory-derived zinc toxicity data for aquatic macroinvertebrates is likely due to unavailability of cultured test organisms and uncertainty associated with field collected organisms. We successfully collected, transported, and acclimated a mayfly (*Drunella grandis*), a stonefly (*Isoptera* sp.), and a case dwelling caddisfly (*Lepidostoma* sp.) from the South Fork Cache la Poudre River, Colorado, a stream unaffected by heavy metals from mining activities. We were unable to run tests with two other collected aquatic insects, the mayfly *Baetis tricaudatus* and the free-living caddisfly *Dolophilodes aequalis*, either as a result of sensitivity to handling stress from collection, transport, and test set up (*Baetis*), or inability to acclimate to laboratory test conditions (*Dolophilodes*). Static-renewal 96-hr acute toxicity tests were conducted in soft, hard, and very hard reconstituted lab water. Differences in toxicity and acclimation to laboratory conditions were observed within and between Orders. *Drunella grandis* performed very well both in the field collection phase and under laboratory conditions (LC<sub>50</sub> >6.29 mg/L in hard water and >3.05 mg/L in soft water). Similarly, *Lepidostoma* sp. performed well (LC<sub>50</sub> >38.8 mg/L in hard water and >81.7 mg/L in very hard water). No acute toxicity was observed for zinc concentrations up to 27 mg/L for *Isoptera* sp. Overall, toxicity values for these field collected EPT taxa were much greater than predictions based on comparisons to other sensitive macroinvertebrates or zinc tolerance values reported from field observations of aquatic insects near inputs of heavy metals (e.g., mining areas). This study demonstrated that with proper handling, and species specific considerations, use of field collected native species may be a valuable tool to help assess toxicity in aquatic environments.

**TP226 Comparing *Anadara trapezia* exposure, dose and response to metal contaminated estuarine sediments using laboratory and field exposures** A. Taylor, University of Canberra; W.A Maher, University of Canberra / Dept. of Health, Design & Science. Establishing relationships between metal exposure, internal dose and associated biological effects for organisms is necessary to understand the fate and effects of metals in the environment. The accumulation and sequestration of biologically available metals by aquatic organisms, particularly bivalve molluscs, has led to their use as biomonitors of metal contamination, as it this portion which is of interest in pollution effects assessments. Biomarker measurements can provide evidence that organisms have been exposed to contaminants at levels that exceed their detoxification and repair capacity, thus establishing the link between contaminant exposure and ecologically relevant effects. Laboratory exposures, in which physiochemical factors can be controlled, using previously unexposed organisms are routinely used in the development of toxicological effects tests. Metal accumulation is influenced by metal bioavailability via water, sediment and dietary pathways. These pathways may be influenced by mode of exposure and the history of previous exposure which in turn may affect biological responses. An approach for establishing the relationships between tissue metal dose and biological response of exposure to copper, zinc, cadmium, lead, and selenium contaminated sediment from Lake Macquarie, NSW, Australia, was evaluated for the sediment dwelling bivalve mollusc *Anadara trapezia*. A comparison of tissue metal accumulation and effects was made between resident organisms, and previously unexposed organisms in laboratory and in-situ caged exposures. Organism dose was measured by total metal tissue burden. Subcellular fractionation of whole tissue was used to determine what fraction of the total accumulated tissue metal was in a metabolically available form. Measurements of oxidative stress (total antioxidant scavenging capacity of cells and lipid peroxidation) were used as effects biomarkers of metal exposure. Lysosomal membrane stability was used as a measure of cellular integrity. By identifying relationships between exposure and effects at various levels of biological organisation, using a variety of exposure approaches, a better understanding of the mechanisms of organism stress responses to metals in ecological systems was gained and the predictive capability of ecological risk assessment improved.

**TP227 Experimental Design Affects Social Behavior Outcomes in Adult Zebrafish Developmentally Exposed to Lead** D. Weber, University of Wisconsin-Milwaukee / Childrens Environmental Health Sciences Center, University of Wisconsin-Milwaukee / Great Lakes Water Institute; J. Ghorai, University of Wisconsin-Milwaukee / Mathematical Sciences. Lead (Pb<sup>2+</sup>) affects neuronal and endocrine systems and, therefore, effects behavioral outcomes, especially social interactions. A chamber in which mirrors were used as a means to stimulate agonistic interactions in adult zebrafish was



designed such that the test subject may or may not have a refuge in which it can escape from the mirror image. Each experimental design was also conducted without any mirror in the test chamber to assess background activity patterns as a function of chamber design and developmental Pb<sup>2+</sup> exposure regimen. After developmental exposures (2-24 hours post fertilization) to Pb<sup>2+</sup> (0-10µM), adult (9-months old) male and female zebrafish were tested separately in each chamber design. In the first design in which the mirror was always in view, i.e., no refuge zone, an increase in percent time spent in the mirror zone and percent time of high mobility at the mirror in both males and females displayed a significant increase over control values at all Pb<sup>2+</sup> exposure regimens. In the second design in which there was a refuge zone where no mirror was in the field of vision, there were no significant differences in percent time spent or mobility level at the mirror within each sex between exposure regimens; significant differences between sexes were evident between treatments. Thus, depending upon the design of the behavioral testing apparatus, very different conclusions could be drawn.

**TP228 Modifying Effects of Temperature on Metal Toxicity to *Lemna turionifera*** T. Crowell, Western Washington University / Environmental Toxicology; C. Murdock, J. Ford, R. Sofield, Western Washington University. Environmental parameters, such as temperature, can have modifying effects on metal bioavailability and toxicity, and on plant physiology. Increases in temperature would be expected to increase metal uptake, resulting in more toxicity. However, growth dilution may occur resulting in less toxicity to plants. The effects of temperature and metals on frond growth, frond chlorosis, total chlorophyll and bioaccumulation in *Lemna turionifera* (duckweed) were investigated through seven day static renewal tests using cultured plants from a local pond that were acclimated for two weeks at 20°C and 28°C prior to exposure to silver, copper, and chromium. Effective endpoints include percent growth, percent chlorotic fronds, chlorophyll content, and accumulated metals concentrations. The percent growth was more affected at lower temperatures for all metals, while percent chlorosis was more affected at higher temperatures for all metals. Percent of the fronds that were chlorotic reached maximum levels in the low temperature exposures at 37.3%, 81.8%, and 77.6% for silver, copper and chromium, respectively. This maximum effect for chlorosis was not identified in the 28°C exposures at the same metal concentrations. Based on the IC50s, at 28°C growth was more inhibited than at 20°C for silver and copper. There was no difference in the chromium IC50s for growth at both temperatures. This study suggests that metal toxicity is modified by temperature in *L. turionifera*. Chlorophyll and bioaccumulation data will be used to explain current results.

**TP229 The Effect of Speciation and Modifying Factors on the Uptake and Accumulation of Selenium in Two Freshwater Plants** B.P. Lo, Nautilus Environmental and Simon Fraser University; J.R. Elphick, Nautilus Environmental; G. Guilron, Cardero Coal; S.A. Hughes, Shell Health; C.J. Kennedy, Simon Fraser University / Department of Biological Sciences. Selenium is essential for the synthesis of seleno-amino acids (ex. selenomethionine and selenocysteine), however, the range between physiological necessity and toxicity is narrow. In aquatic environments, inorganic selenium is typically present at concentrations well below those that can result in biological effects, therefore risk to aquatic organisms is primarily associated with bioaccumulation through the food chain. To better characterize the uptake of selenium into freshwater food chains, we explored the roles of speciation and modifying factors on the uptake of inorganic selenium into a freshwater algae (*Pseudokirchneriella subcapitata*) and a floating aquatic macrophyte (*Lemna minor*). These species were exposed to a range of selenate and selenite concentrations in waters with a range of sulphate concentrations to explore the relationship between sulphate concentration and selenium uptake. Furthermore, the effect of two other anions, nitrate and chloride, on selenite uptake was also investigated. The results of this study demonstrate that selenium speciation and modifying factors, such as elevated sulphate concentrations, may play a significant role in regulating the uptake and accumulation of selenium into the lower trophic levels of freshwater food chains, and provides useful information for predicting the uptake of selenium under differing water quality conditions.

**TP230 A chronic toxicity test of anthracite ash from a thermo-electric power plant using midge and cladoceran species** H. Mo, Korea University / Division of Environmental Science and Ecological Engineering; Y. Kim, Korea University / Division of Environmental Science and Ecological Engineering, College of Life and Environmental Sciences; Y. Bae, Korea

University; J. Kim, Seoul National University; C. Kim, Korea Urban Forestation Co., Ltd.; K. Cho, Korea University / Division of Environmental Science and Ecological Engineering, College of Life and Environmental Sciences, Korea University / # 407, Division of Environmental. Thermo-electric power comprises a third of power production around the world, and the amount of anthracite used for thermo-electric power is very large because anthracite is relatively cheaper than any other fossil fuels. Recently thermo-electric power plant is becoming more important after nuclear crisis in Japan in 2011. However, mass amount of anthracite ash from thermo-electric power is produced inevitably. The entire ash produced is dumped in landfill because sea disposal is prohibited by law in South Korea. But, if the ash slurry has toxicity the leachate can be harmful to ecosystem. Therefore the objective of this study is to assess the chronic toxicity of anthracite ash slurry on midge and cladoceran test species. Especially the result of this test would be important to debate that the use of anthracite ash to make an artificial wetland.

**TP231 Acute Toxicity of Commercial Heat Transfer Fluids to Freshwater Fish and Invertebrates** D.G. Poirier, R. Chong-Kit, Ontario Ministry of the Environment / Laboratory Services Branch; J. Sowa, University of Guelph; W. Lusk, Ontario Ministry of the Environment / Technical Support Eastern Region; M. Nowierski, Ontario Ministry of the Environment / Standards Development Branch; T. Watson-Leung, Ontario Ministry of the Environment / Aquatic Toxicology Unit, Ontario Ministry of the Environment / Aquatic Toxicity Scientist, Ontario Ministry of the Environment / Sediment Toxicologist. The provincial government of Ontario is promoting an increase in the use of alternative energy sources to reduce reliance on coal generated power, provide energy sustainability and reduce potential sources of air pollution. An attractive option is the use of geothermal energy to heat homes and businesses, particularly in rural areas of the province. The most common types of earth energy systems are sub-surface systems, with lake loop (or submerged) systems now becoming more common. Both sub-surface and lake loop systems have heat exchange loops which are filled with heat transfer fluids (HTFs). With respect to sub-surface systems, there is potential for HTF leakage into groundwater, where contaminated groundwater could reach surface water. With respect to submerged systems, there is potential for these systems to be damaged by winter ice, boating hazards and wave action, causing HTFs to escape into the waterways. Spills can also occur during the installation of the geothermal systems. To understand the potential impacts on aquatic organisms from these HTFs, and to provide guidance to manufacturers and installers of these materials, the Aquatic Toxicity Unit of the Ontario Ministry of the Environment (OMOE) conducted acute toxicity testing on seven aquatic species (five invertebrates and two cold water fish) with five common formulated HTFs and their base components (propylene glycol and denatured ethanol). Toxicity test design adhered to national or international standardized methods where available, and best practices when standard methods were not available. The invertebrates tested were *Daphnia magna*, *Chironomus dilutus*, *Hexagenia limbata*, *Lumbriculus variegatus* and *Hyalella azteca*. Fish tested were Rainbow Trout (*Oncorhynchus mykiss*) and Lake Trout (*Salvelinus namaycush*).

**TP232 Are Creosote Pilings Hazardous to Herring Embryos?** D. Duncan, University of Alaska Fairbanks / School of Fisheries and Ocean Sciences, University of Alaska, Southeast; S. Rice, NOAA NMFS Auke Bay Laboratory; M. Stekoll, University of Alaska Fairbanks; M. Carls, NOAA NMFS Auke Bay Laboratory; R. Perkins, University of Alaska Fairbanks. Creosote treated wood is a building material for docks, harbors, and other marine structures in Alaskan waters. Creosote is manufactured from distilled coal tar, contains up to 85% polycyclic aromatic hydrocarbons (PAH), and functions both as a wood preservative and a pesticide. Chronic leakage of PAHs may be harmful to essential fish habitat, particularly for early life stages. Pacific herring (*Clupea pallasii*) spawn nearshore on vegetation such as sea grasses, algae, and other substrates, including on or near piling structures. This poster reports on the lab component of our study to determine the toxicity of leachate from creosoted wood to herring embryos. These levels will be compared to environmental levels of PAH from existing pilings in the Juneau area. Pacific herring embryos from 21 different females were exposed to seven doses of creosote treated wood effluent under controlled laboratory conditions. Dose levels of PAH, monitored by GCMS, ranged from 0.14 to 26 ppb PAH. After 15 days of exposure, slides with eggs attached were removed from exposures and allowed to hatch over the next few days. Skeletal deformities and reduced swimming ability were the most sensitive

indicators of effects. The 50% effective concentration (EC50) for creosote derived PAH is approximately 16 ppb. Skeletal deformities and reduction in swimming ability as a result of PAH exposure in teleost fishes are known to have negative effects on long term survival. The question remains if these dose levels are ever achieved in the environment near pilings. This field study is currently in progress.

**TP233 Assessing the mechanisms of toxicity in mountaintop removal/valley fill coal mining-affected watershed samples using a *Caenorhabditis elegans* bioassay** E.A. Turner, Duke University; M.C. Arnold, Duke University / Nicholas School of the Environment, Duke University; J.N. Meyer, Duke University / Nicholas School of the Environment. Though mountaintop removal/valley fill mining (MTR/VF) has become increasingly prevalent in Appalachia, there are relatively few lab-based studies investigating the toxicity of mine-impacted water and stream sediment. We have sampled sites on the Mud River and its tributaries chosen on the basis of GIS analysis as part of an interdisciplinary study aimed at testing for contaminants and toxicity in stream waters and sediments in areas affected by MTR/VF. Using *Caenorhabditis elegans* as an ecotoxicological model, we developed a growth assay to assess the potential toxicity of water and sediment pore water samples. In addition to wild-type nematodes, knockout strains were selected for the purpose of investigating the mechanisms of toxicity. The relative role of heavy metals and osmotic stress were investigated using three different deletion mutants. *Pcs-1* and *smf-2* knockout mutants were used to assess the contribution of metals to toxic growth effects, and the role of osmotic stress was investigated using *gpdh1;2* double knockout mutants. Nematodes were exposed for 3 days to a dosing solution consisting of 50% stream water or 50% sediment pore water and 50% EPA-defined reference water. Ultraviolet C radiation-treated *Escherichia coli* were used as a food source to reduce potential confounding effects of bacterial metabolism. Growth of the nematodes was measured with a COPAS biosort, using length and optical density as metrics. Our data show that both stream water and sediment pore water from the Mud River and its tributaries produce significant effects on the growth of both wild-type and mutant *C. elegans*. *Pcs-1*, a mutant strain that lacks the enzyme needed to produce the heavy-metals chelating protein phytochelatin, was the most sensitive to sediment pore water, with treated worms demonstrating impaired growth in comparison to untreated controls and to treated wild-type nematodes. *Gpdh1;2* mutants lacking both glycerol-3-phosphate dehydrogenases responsible for synthesis and accumulation of glycerol in response to osmotic stress were more sensitive to stream water than their wild-type counterparts. Collectively, growth data from wild-type and mutant nematodes suggest that osmotic stress is the principle mechanism of toxicity in the water column, whereas metals are the most relevant toxic element in the sediment pore water.

**TP234 Bioaccumulation and food chain dynamics of selenium at the Kingston coal ash spill site** R.R. Otter, Middle Tennessee State University / Biology, Middle Tennessee State University / Department of Biology, Middle Tennessee State University / Assistant Professor; F.C. Bailey, Middle Tennessee State University / Department of Biology; M. Hayden, Middle Tennessee State University / Biology; A. Fortner, Arcadis US; T. Mathews, Oak Ridge National Laboratory / Environmental Sciences Division, Oak Ridge National Laboratory / Biological and Environmental Sciences Division; M. Adams, Oak Ridge National Laboratory. On December 22, 2008 a dike containing coal fly ash from the Tennessee Valley Authority's Kingston Fossil Plant near Kingston, Tennessee USA failed and resulted in the largest coal ash spill in U.S. history. Coal ash, a by-product of coal combustion, is known to contain multiple contaminants of concern, including selenium. The purpose of this study was to investigate the bioaccumulation and food chain dynamics present at ash-associated sites compared to reference sites. Analysis of selenium burdens in organisms spanning multiple trophic levels was included to determine how selenium is incorporated into the aquatic food web. Selenium concentrations in periphyton and snails showed no significant differences between ash-associated sites and reference sites, but concentrations in fish muscle tissue (bluegill and redear sunfish) were significantly higher at ash-associated sites with respect to reference sites. Of all fish species considered in this study, redear sunfish showed the highest bioaccumulation of selenium with an average muscle concentration of 5.0 mg/kg dw. Stable isotope analysis ( $\delta^{15}\text{N}$  &  $\delta^{13}\text{C}$ ) showed a positive relationship between selenium bioaccumulation and  $\delta^{15}\text{N}$  indicating an increased bioaccumulation of selenium as trophic level increases.

**TP235 Challenges Faced by Wildlife Operations During the Yellowstone River Oil Spill** K.J. Nelson, U.S. Fish and Wildlife Service / Environmental Contaminants Program; D.R. Rouse, U. S. Fish and Wildlife Service / Environmental Contaminants Program. On July 1, 2011, approximately 64,000 gallons of crude oil spilled into the Yellowstone River from a ruptured 12-inch pipeline. The river was at flood stage and the released oil was deposited throughout the floodplain. During high flows, the ability to look for oiled wildlife was limited because of the dangerous flood conditions on the river. Numerous bird species were nesting along the river corridor at the time of the spill including bald eagles, great blue herons, and osprey. Protection of nests from response disturbance was one of many priorities of Wildlife Operations. Large woody debris piles, common in large, undammed river systems, had captured oil during the spill, and as floodwaters receded, began releasing oil back into the river system. Wildlife using the debris piles were often exposed to the associated oil pools, or from direct contact with oiled woody debris. Wildlife oiled in these debris piles included birds, frogs, toads, and snakes. Implementation of wildlife priority areas for cleanup, such as large woody debris piles, helped reduce wildlife exposure to oil.

**TP236 Changes in toxicity of the leachate from the residues by low-temperature pyrolysis of industrial wastes** N. Ota, R. Shoji, Tokyo National College of Technology. Waste treatment is a very serious problem all over the world these days. For example, incineration technology, which is widely used, is being disputed at present because it causes many problems such as producing dioxins and discarding heavy metals. Low-temperature pyrolysis is a technology to make industrial wastes stable in the environment and to reduce their volume. In this study, chemical and ecotoxicological data of the leachates from the residues by different reaction times of the low-temperature pyrolysis of industrial wastes were examined. The residues were analyzed for their leaching properties by using the Japanese Leaching test 13 [JLT13]. The ecotoxicological assay was performed using fresh water alga *Pseudokirchneriella subcapitata* [*P. subcapitata*] growth inhibition assays for 72 hour of exposure time according to the OECD [Organisation for Economic Co-operation and Development] ecotoxicity test guideline 201, and fresh water crustacean *Daphnia magna* [*D. magna*] swimming inhibition assays for 48 hour of exposure time according to the OECD ecotoxicity test guideline 202. Each toxicity test was carried out with changing in concentration of the leachate solution. In the leaching tests, the concentration of some metals decreased as pyrolysis reaction going on. In the ecotoxicity tests, the lowest level of the ecotoxicity was observed in Ash 3 that was taken from long-term pyrolysis treatment among three kinds of the residues. Additionally, an effect of salt concentration in the leachate on the growth inhibition of algae was significant. It is suggested that the effect of salt concentration on the growth inhibition of algae must be considered in the assessment of toxicity of the leachate from residues.

**TP237 Collect It or Lose It: Harnessing Elusive Ephemeral Data** M.J. Anderson, California Department of Fish and Game / Office of Spill Prevention and Response; M. Ammann, Chevron / Chevron Energy Technology Company; B.M. Joab, CA Dept. of Fish and Game, Office of Spill Prevention and Response / Office of Spill Prevention and Response. The initial hours of an oil spill response are often chaotic as the response team transitions from an emergency response to management of a project. During this transition period, there is an opportunity to collect critical, time sensitive environmental information that, if not collected, will be forever lost. Such data are referred to as "ephemeral data", and are especially important in the subsequent assessment of injuries to natural resources during the Natural Resources Damage Assessment (NRDA). Trying to complete an ephemeral data collection plan during an actual spill response is sure to result in a missed opportunity, not to mention the loss of information that has important implications for the NRDA. Natural Resource Trustees (California Department of Fish and Game, Office of Spill Prevention and Response, the National Oceanographic & Atmospheric Administration, the US Fish & Wildlife Service, and the Bureau of Land Management) in California and Chevron have jointly completed a project to prepare an Ephemeral Data Collection Plan (EDCP) for Chevron's marine terminal located in Eureka, CA. The EDCP includes information on sampling media (e.g., water, sediment, and biological tissue), sampling and analytical protocols, sampling locations with GPS coordinates, and key contacts such as first responders, consultants, and laboratories. The EDCP also includes photographs of sampling locations to help orient members of the field sampling team. Because of the swift tides and currents in Humboldt Bay, and other considerations

that could impede the successful implementation of the EDCP, Chevron voluntarily agreed to collect ambient samples of sediment, water, and tissue for chemical analysis of polynuclear aromatic hydrocarbons (PAHs), as described in the EDCP, to help establish a pre-spill reference condition.

**TP238 Comparison of Arctic and Non-Arctic Marine Species Acute Sensitivities to Physically and Chemically Dispersed Petroleum** W. Gardiner, NewFields LLC; J.Q. Word, NewFields / Applied Environmental Sciences, NewFields LLC / Applied Environmental Sciences.

The toxicity of oil and dispersed oil on epipelagic and pelagic organisms in the Arctic is a critical component in evaluating the net environmental benefits of response alternatives to spills in open waters. While there is a substantial body of literature for sub-arctic, temperate, and tropical species, current toxicity data available for arctic species is limited. The applicability of toxicity data for non-arctic species is uncertain, in part because polar species have developed specialized adaptations to cope with the stresses from these harsh environmental conditions. The purpose of this study was to evaluate the relative sensitivity of Arctic species to non-arctic species to petroleum and dispersed petroleum. Median-lethal concentration data for total petroleum hydrocarbons (TPH) and parent naphthalene in physically and chemically dispersed petroleum were used to construct species sensitivity distributions (SSD) for arctic and non-arctic species. Data were limited to acute toxicity tests conducted with spiked-exposures. The sensitivity of individuals were remarkably similar for the arctic and non-arctic species groups. For both the WAF and parent naphthalene, the SSDs were overlapping, with calculated HC5 and HC50s within a factor of 2. The largest difference in regional sensitivity was observed in the CEWAF, where the SSD was shifted to the right indicating that the non-arctic species were more sensitive than the arctic species. However, when oil type and differences in TPH measurement were taken into account, there was little difference in the sensitivity to CEWAF for the two regional groups. The acute toxicity of the parent naphthalene contained in declining exposures was remarkably similar for both chemically and physically dispersed oil tested with species from arctic, temperate, and tropical regions with a variety of oil types.

**TP239 Comparison of exposure methods and toxicity of several heavy fuel oils to fish embryos** J.W. Martin, Trent University / Division of Analytical and Environmental Toxicology; J. Adams, Queen's University / Biology; P.V. Hodson, Queen's University / School of Environmental Studies.

Small-scale oil spills occur frequently due to transportation of oil products. Heavy oils in particular are transported in large quantities. A spill of such an oil occurred in August 2005 when nearly 150 000 L of No. 6 heavy fuel oil was spilled into Wabamun Lake (Alberta, CA) when a train derailed. Bunker C is a residue of refining crude oil and a very complex mixture containing polycyclic aromatic hydrocarbons (PAH), particularly the 3-4 ringed alkylated forms that cause sub-lethal toxic responses in early life stages of rainbow trout (*Oncorhynchus mykiss*). Static daily renewal tests with heavy fuel oil coated on glass plates (artificial substrates) demonstrated greater oil toxicity (as mg added oil/L) to trout embryos than oil that was mechanically or chemically dispersed. Nearly four years after the spill at Wabamun Lake, oil patches still persist in near-shore sediments where fish spawn. The toxicity of oil that sinks and persists in sediments is not well understood because the mechanisms of exposure are not characterized. The objective of this research is to assess how the behaviour of heavy oil in water interacts with exposure and toxicity to early life stages of fish. Specifically, mechanical and chemical dispersions were compared to flow-through oiled gravel columns for several heavy oils and a reference crude oil to assess whether the toxic constituents of heavy oil are transferred to water quickly enough to cause toxicity. Dose-dependent mortality and signs of sub-lethal toxicity were observed in embryonic trout exposed to both chemical dispersions and to the outflow of the oiled gravel columns. Overall, heavy oils were more toxic to trout than medium crude oil and oiled gravel dosing systems were more toxic than traditional dispersions, coincident with concentrations of alkyl-PAH. Overall, this research indicates that heavy oils that are prone to sinking are quite toxic and, on sediments, produce long-term risks to fish embryos.

**TP240 Compensating for natural resource injury: a restoration trifecta** P.A. Lebednik, L.W. Paz, R.E. Moniz, P.R. Martos, M. Barkley, Arcadis US.

Restoration projects are typically implemented to compensate for natural resource injury as a result of releases of oil or hazardous substances. When innovative restoration design is employed, such projects may generate

multiple natural resource and human use benefits within a remarkably short period and with little or no added cost. One such highly successful project will be presented as a case study in multiple-benefit restoration. A release of product from a petroleum pipeline in California's Suisun Marsh resulted in contamination of marsh sediment and exposure of marsh biota. Following response, cleanup and natural resource damage assessment, restoration of onsite uses was implemented. Restoration approaches were tailored to site and exposure conditions. Based on a net environmental benefit analysis (NEBA), areas that experienced relatively low exposure and injury were passively restored. Areas that experienced greater exposure were actively restored. The presentation will focus on the actively restored portion of the site and will describe how substantial compensatory benefits for an endangered species, tidal wetland habitat, and waterfowl recreational use were incorporated into design criteria to produce a fully integrated restoration plan. The most challenging design task, 3D spatial accommodation, will be discussed. Site construction involved infrastructure enhancement, major earthwork, soil importation and precision contouring. Following the construction phase, development of habitat types was spatially consistent with the restoration blueprint. Less than three years after construction, restoration of the site was deemed highly successful by natural resource and regulatory agencies as well as recreational users. The functionality of the restored site was enhanced in comparison with the pre-release condition.

**TP241 Contaminants of Emerging Concern in the Great Lakes Basin: A Spatial Interpretation of Sediment and Water Chemistry Data** S. Choy, J. Banda, D. Gefell, Z. Jorgenson, U.S. Fish and Wildlife Service; K. Lee, U.S. Geological Survey; J. Moore, U.S. Fish and Wildlife Service.

Despite growing concerns about the presence of contaminants of emerging concern (CECs) in the environment, we currently have a tenuous understanding of CECs with respect to sources, routes of exposure, and impacts to fish and wildlife. The U.S. Fish and Wildlife Service (USFWS), in collaboration with the U.S. Environmental Protection Agency (USEPA) and U.S. Geological Survey (USGS), has initiated an early warning program to detect and identify CECs and their effects to fish and wildlife. Our investigation focuses on tributaries of the Great Lakes Basin, with an emphasis on Areas of Concern (AOCs). In conjunction with fish sampling, surface water and sediment samples were collected in 7 AOCs. Field samples were analyzed by the USGS National Water Quality Laboratory in Denver, CO for over 150 select CECs, including pharmaceuticals, hormones and wastewater indicators. Using Geographic Information System (GIS) technology to map water and sediment chemistry data, land use and suspected point and non-point sources of CECs, we investigated patterns in the distribution, combinations, and concentrations of CECs. The results will contribute to our understanding of the sources and routes of exposure of CECs and will be correlated with fish bioindicator assessments to determine if there are relationships between chemical compositions and effects in fish. Presented here is sediment and chemistry data and an interpretation of the spatial distribution.

**TP242 Effect of cold plasma treatment on water and its evaluation by using *Daphnia magna*** T. Abe, Tsuruoka National College of Technology / Dept. of Chemical and Biological Engineering, Tsuruoka Nat. College of Technology / Dept. of Material Engineering; H. Gandou, H. Yoshiki, Tsuruoka National College of Technology.

Recently, atmospheric-pressure nonequilibrium plasmas called "cold plasmas" have received much attention in view of their environmental and bio-medical applications. In cold plasma, various radical species such as atomic oxygen and OH are generated and these radicals play an important role in degradation of toxic organic compounds and sterilization of bacteria and fungi. So, many kinds of cold plasma devices have been developed so far. In this study, the cold plasma generated in contact with water was used to chlorophenols degradation and the influence of the plasma treatment was evaluated by means of the *Daphnia magna* acute immobilisation test. The cold plasma irradiation on the M4 medium which is the breeding water for *D. magna* was also conducted to check the toxic effect of plasma treatment. *D. magna* immobilisation test was carried out based on OECD Guidelines for the Testing of Chemicals (TG202). 20 neonates within after the birth 24 hours exposed with metal ions under static condition at constant temperature  $20 \pm 2$  degree C. The neonates were observed at 24 and 48 hours after exposure and recorded the number of survived neonates. Additionally, cold plasma degraded indigo carmine, which indicated a proportion of irradiation time to reduction of indigo absorbance. These results showed that cold plasma degraded some chemicals and it was valuable to evaluate cold plasma with *D. magna*.



**TP243 EPA's Great Lakes Human Health Fish Tissue Study** L.L. Stahl, US EPA, Office of Water / Office of Science and Technology; J.B. Wathen, U.S. EPA Office of Water, Office of Science and Technology / Office of Science and Technology, U.S. EPA, Office of Water / Office of Science and Technology; E.W. Murphy, J. Fisher, E. Smith, US EPA, Great Lakes National Program Office / GLNPO; A.R. Olsen, U.S. EPA Office of Research and Development / Western Ecology Division, National Health and Environmental Effects Laboratory; B.D. Snyder, Tetra Tech, Inc. / Center for Ecological Sciences. EPA's Office of Water, Great Lakes National Program Office, and Office of Research and Development are collaborating to conduct the Great Lakes Human Health Fish Tissue Study. This is the first statistically based study of fish contamination in the Great Lakes, and it adds a human health component to the ecological assessments EPA is conducting under the statistically designed National Coastal Condition Assessment (which includes the Great Lakes). Assessment of contaminants in Great Lakes fish for human health applications involves collecting one fish sample from 157 randomly selected nearshore sites (depths up to 30 m or distances up to 5 km from shore) throughout the five Great Lakes (about 30 sites per lake). Fish samples consist of similarly sized adult fish of the same species that are commonly consumed by humans and optimally contain five specimens. Fillet tissue from each sample is being analyzed for mercury, 13 perfluorinated compounds or PFCs (including perfluorooctanoic acid or PFOA and perfluorooctanesulfonic acid or PFOS), 52 polybrominated diphenyl ether congeners or PBDEs, and the full suite of 209 polychlorinated biphenyl congeners or PCBs. EPA is also including analysis of five omega-3 fatty acids in fish fillets (including alpha-linolenic acid or ALA, eicosapentaenoic acid or EPA, and docosahexaenoic acid or DHA) to obtain species-specific data on these compounds. This will address an existing data gap and identify fish with higher levels of omega-3s and potentially greater health benefits. EPA collected the fish samples for this study from mid-May through mid-November in 2010 and received some support from Great Lakes states for this sample collection effort. EPA anticipates having results available to report in 2013.

**TP244 Estuarine Sediment Contamination – Assessment and Management** P.M. Chapman, Golder Associates Ltd.; F. Wang, University of Manitoba / Centre for Earth Observation Science, Department of Environment and Geography, University of Manitoba; S. Caeiro, Universidade Aberta / Departamento de Ciências e Tecnologia; A. Del Valls, Universidad de Cadiz / Departamento de Química, Física, Facultad de Ciencias del Mar y Ambientales. Sediment contamination remains a global problem, particularly in estuaries which are the recipients of chemicals from multiple near- and far-field sources. Although estuaries (including coastal lagoons) are highly productive ecosystems, approaches for assessing and managing sediment contamination are not as well developed as in marine and fresh waters. This presentation will begin by describing the estuarine environment and the current relevance of Remane's "paradox of brackish water", then proceed to describe and discuss the role of the following assessment techniques relative specifically to the changing estuarine environment: chemistry; individual and multi-species toxicity tests; biological surveys; bioindicators; biomarkers of exposure and effect; and, integrative assessments. A decision-making framework following assessment of sediment contamination that requires management action will be described, focused on ecosystem services. Major uncertainties and the key research that could assist in resolving those uncertainties will also be described.

**TP245 Evaluating fish health after an inland oil spill** M. Carney, Stratus Consulting Inc.. Adult and juvenile fish can be sensitive to toxic components of oil such as polycyclic aromatic hydrocarbons (PAHs). This presentation describes different methods that can be used to quantify exposure and toxicity of PAHs in fish in river systems exposed to spilled oil. Methods described include: observations of gross abnormalities, histopathology, immunohistochemistry, virology, hematology, chemical analysis of bile and other tissues, analysis of P450 mRNA, EROD assays, DNA adduct assays, and otolith microchemistry. Sample methods, timing, storage, and analysis considerations are discussed.

**TP246 Evaluating the contribution to toxicity of weak black liquor in bleached kraft pulp mill effluents** R.L. Ragsdale, NCASI / Aquatic Biology, NCASI; R.M. Sofield, Western Washington University / Huxley College of Environment; T. Bousquet, NCASI; D.L. Cook, NCASI / Department of Chemistry; W. Streblow, J. Ikoma, S. Stratton, C. Flinders, NCASI.

Exposure of conventional marine and freshwater toxicity test organisms to pulp and paper mill effluents sometimes elicits a biological response, with the causative agents largely unknown. We evaluated the contribution of weak black liquor (WBL) to toxicity by adding various concentrations of WBL (0 [control] to 0.527g/L) to untreated wastewater from each of seven bleached kraft mills and subjecting these to laboratory biotreatment in aerobic reactors. Following laboratory biotreatment, chronic toxicity of the simulated effluents were evaluated using 48-h *Mytilus galloprovincialis* embryo-larval development (EC50) and 7-d *Ceriodaphnia dubia* reproduction (IC25) toxicity tests. The treated wastewater control and WBL-spiked biotreated effluents were characterized for chemical constituents. Correlation analysis was used to determine the relationship between: 1) WBL solids and effluent chemical parameters; 2) WBL solids and biological response; 3) measured effluent constituents and biological response; and 4) the relative sensitivity of the two test organisms. Multivariate methods (cluster analysis and PCA) were also used to explore data patterns, and to identify effluent chemical parameters that might relate to WBL solids or effluent toxicity. For all laboratory biotreated effluents, color and concentrations of DCOD, polyphenols,  $\beta$ -sitosterol, and three resin acids (palustric, abietic, and neo-abietic) increased with increasing WBL solids. Embryo-larval development of *M. galloprovincialis* and *C. dubia* reproduction decreased significantly with increasing WBL solids, with *M. galloprovincialis* embryo-larval development a more sensitive endpoint than *C. dubia* reproduction. For both test organisms, toxicity was most variable across mills in the wastewater control (EC50=6.1-22.0, IC25=35.5-100) and greatest in the highest WBL treatment (EC50=1.8-3.6, IC25=16.7-60.1). Negative correlations were found between the EC50 and some effluent constituents (color, conductivity, polyphenols, hardness, DCOD, DOC, and abietic acid). None of the measured chemical parameters correlated with chronic toxicity to *C. dubia*. Clustering and PCA showed that simulated effluents were most similar based on mill origin, rather than factors such as chronic toxicity or WBL solids. Study findings suggest that WBL solids contribute to the biological response to effluent exposure, and lend support to the development of best management practices to reduce WBL losses to mill wastewater.

**TP247 Evaluation of the water quality of the Corumbataí river (São Paulo – Brazil) through toxicity test with *Ceriodaphnia dubia*** L.A. Maranhão, M. Inafuku, Center for Nuclear Energy in Agriculture/University of São Paulo; R.G. Botelho, University of São Paulo / Ecotoxicology; G. Moura Andrade, L. Figueiredo, V. Tornisiello, Center for Nuclear Energy in Agriculture/University of São Paulo. The aquatic environment is the ultimate recipient of an increasing amount of contaminants as a result of the discharge of industrial, agricultural and urban wastes. This study was made in the Corumbataí river since it supplies the Piracicaba city and all activities measured above do part of the reality of this hydric course. During one year the quality of this river was evaluated monthly in six points of sampling through physical chemical parameters (pH, water temperature, conductivity, dissolved oxygen) and chronic toxicity tests with the microcrustacean *C. dubia*. For each sampling points of water were used ten replicates with one organism in each as well as the control group (reconstituted water). The test period was seven days and the parameters evaluated were mortality (acute effect) and reproduction (chronic effects). The tests solutions (without dilution) were renewed each 2 days and the organisms were fed with *Pseudokirchneriella subcapitata*. At the end of test the means of reproduction were compared with the control group by tukey test. In all months the sampling points pH and water temperature were within the values established by the Brazilian environmental legislation differently dissolved oxygen and conductivity. No acute effects were observed. In the chronic toxicity tests 50 samples analyzed (of 72) presented toxicity when compared with the control group. Can be concluded that the evaluation with *C. dubia* showed chronic effects of the Corumbataí river water.

**TP248 Genome expression response to experimentally-weathered south Louisiana crude oil in gulf killifish profiled across tissues, doses, and time** W. Pilcher, Louisiana State University; G. Mayer, Texas Tech University; A. Whitehead, Louisiana State University. Almost five million barrels of south Louisiana crude oil was released into the environment following the explosion of the Deepwater Horizon oil platform on April 20, 2010, and effects on native species are not well known. A previous field study, completed in 2010, tracked genome expression responses in native killifish resident in northern Gulf of Mexico marshes, including one that was directly oiled. Those data suggested significant biological effects from

contaminating oil that persisted over time. As a companion to field studies, we sought to determine the genome expression response of the same native killifish to oil under controlled laboratory conditions. A dose-response and time-course experiment was conducted using experimentally-weathered surrogate oil to further characterize the transcriptional response, and to test for additional impacts on health and performance. Transcriptome-wide gene expression responses were determined from gill and liver tissues, across a range of doses, and throughout a time-course of exposure, to experimentally weathered surrogate oil. Dose and time course experiments offered insight into biological mechanisms of response. Transcriptional responses to oil in the laboratory were predictive of the transcriptional response observed in the field study that coincided with the timing and location of oil contamination. These responses included increased expression in the genes activated by the aryl hydrocarbon receptor signaling pathway, including phase 1 and phase 2 metabolism genes, among others. Additionally, network and pathway analyses implicated effects on circulatory system processes, apoptosis regulation and reproductive development, and toxicology functions such as cholestasis, steatosis, hyperplasia and carcinoma were implicated in the liver. These genome expression profiles offer additional scope for interpreting genome expression responses observed in the field, and offer additional insight into consequences of oil exposures in this important native Gulf coast species.

**TP249 Histopathology and PAH Concentrations in Peanut Menhaden: Effects of the DeepWater Horizon Oil Spill** C.S Bentivegna, Seton Hall University / Department of Biological Sciences, Seton Hall University / Biology department; K.R. Cooper, Rutgers, the State University of New Jersey / Department of Biochemistry and Microbiology; G.M. Olson, R.J. Portier, Louisiana State University. A blowout of the riser pipeline on the Transoceanic *DeepWater Horizon* (DWH) oil rig at the British Petroleum (BP) Macondo-1 site in the Gulf of Mexico (GOM) resulted in the largest oil spill in U.S.A history. The purpose of this project was to investigate the impact of the 2010 DWH oil spill by monitoring the pelagic, filtering feeding fish, menhaden, both in the GOM and along the Atlantic coast. More specifically, young of the year (peanut menhaden) were collected from the estuaries of Barataria Bay, LA and Delaware Bay, NJ in fall 2010. Barataria Bay was one of the most heavily oil areas during the DWH spill. Fish in Delaware Bay, NJ have been exposed to urban sources of PAHs but not a recent large oil spill. The endpoints included polycyclic aromatic hydrocarbon (PAH) concentrations in digestive tissue as detected by fixed emission fluorescence spectroscopy (FEFS), in whole body tissue as detected by gas chromatography-mass spectrometry (GCMS) as well as levels of tissue damage determined by histopathology. Whole body analysis of peanuts by GCMS found limited types of PAHs. Alkylated phenanthrenes (C2 and C3) ranging from 1.22-6.52 ng/mg were found in fish from both NJ and LA. Larger PAHs including benzo[b]fluoranthene and benzo[a]pyrene were only found in LA fish and ranged from 0.34 to 0.06 ng/mg. FEFS found much higher levels of PAH-like compounds in peanut digestive tissue. Concentrations of naphthol-like PAHs were similar between locations, approximately 5800 ng/mg, while hydroxypyrene-like PAHs were lower in LA compared to NJ fish (129±38 and 186±32 ng/mg, respectively). This resulted in a significantly higher naphthol to hydroxypyrene ratio in LA fish, which has been associated with petroleum exposure. Histopathological data showed severe gill and digestive tissue damage in LA compared to NJ fish. Extensive damage to the gallbladder with ductal dilation, liver necrosis and hemosiderin deposits was found in LA fish. LA fish had moderate to extensive areas where the primary lamellae of gill were fused, which reduces gas exchange and causes stress to the fish. Results clearly showed the types of tissue damage associated with exposure to petroleum and this finding was supported by tissue PAH analysis.

**TP250 Important Considerations Governing the Behavior of Chemicals in Sediments and Water in Tropical and Temperate Environments** R. Wenning, ENVIRON; L.B Martello, ENVIRON International Corporation / Department of Health Sciences, ENVIRON / Department of Health Sciences; K. Leigh, ENVIRON. The behavior of organic compounds and metals in surface waters and, particularly, sediments in tropical regimes has not been well studied, despite evidence suggesting differences in persistence, bioaccumulation, and speciation of metals and organic substances as compared to sediments in colder climates. Several metals and organic chemicals elicit different biological responses in aquatic bioassays and have different environmental fate properties in temperate and tropical environments. In tropical watersheds, it has been shown that the relative flux of

organochlorine insecticides, such as HCH and DDT, in the aquatic environment is smaller than the amount volatilized to the atmosphere. For some fungicides and agricultural chemicals, comparisons of bioassay data suggest that it is often not appropriate to extrapolate fate and toxicity threshold data generated from temperate bioassay studies to evaluate impacts on organisms and ecosystems in tropical areas. Nonetheless, there has been a tendency by regulatory agencies in several countries to adopt sediment quality guidelines – particularly from North America – without adequate consideration of environmental differences that might make such guidelines more or less relevant. In this presentation, the challenges associated with understanding the environmental behavior and risks of different classes of contaminants in waterways and sediments in tropical and non-tropical regions are reviewed. If a temperature-dependent relationship exists, then regulatory approaches and criteria need to take this into consideration. The purpose of this presentation is (a) to consider the potential that there is a fundamental difference in environmental fate and effects characteristics of organic and metal contaminants in tropical vs. temperate environments, and (b) to consider the implications of this for risk assessment and risk management, especially in terms of regulatory sediment quality values. The presentation focuses on physico-chemical properties, temperature, leaching behavior, bioaccumulation, speciation, rates of degradation, and the influences of sediment geotechnical properties such as organic carbon content, pH, anoxic/oxic conditions. It is evident that additional research is needed to better understand the fate and toxicity of relatively common industrial chemicals and environmental contaminants in tropical environments.

**TP251 Integrated approach in order to assess marine sediment quality of areas affected by wastewater discharges: Validation of established marine bioassays** L.A Maranhão, University of Cadiz / Department of Physical Chemistry; M. Garrido-Perez, Andalusian Center for Marine Science and Technology (CACYTMAR), University of Cadiz; T. DelValls, University of Cadiz / Department of Physical Chemistry; M. Martin-Diaz, University of Cadiz. Municipal wastewaters constitute a major pollution source in the aquatic environment, being composed by a mixture of metals, pesticides, altered loads of nutrients, hydrocarbons and other organic compounds as pharmaceuticals and personal care products. Such contaminants are sunk into the sediment which may endanger the aquatic organisms living in the vicinity of urban areas. The acute toxicity of sediment affected by wastewater treatment plant (WWTP) of five cities (Chiclana de la Frontera – P1, Puerto Real – P2, Cádiz – P3, El Puerto de Santa María – P4 (La Puntilla) and P5 (Puerto Sherry) and Rota – P6 control site) in the bay of Cádiz (SW, Spain) was characterized using a battery of bioassays. Whole sediment samples were assessed applying the bioluminescence inhibition of the bacteria *Vibrio fischeri* (Microtox Basic Solid Phase Test) and mortality rate of 10-day amphipod *Ampelisca brevicornis*. Acute toxicity from elutriates samples were evaluated by the bioluminescence inhibition of the bacteria *Vibrio fischeri* (Microtox® Basic Test), the growth rate of the marine microalgae's *Isochrysis galbana* and *Tetraselmis chuii* and the abnormal development of the sea-urchin larvae *Paracentrotus lividus*. Concerning the whole sediment, it was possible to classify the toxic samples according to the Microtox® Basic Solid Phase: P4 > P2 > P1 > P3. Mortality of amphipods was significantly different from the control for P2 and P4. For elutriate samples, Microtox® Basic Test might represent an initial disruption in homeostasis within the bacterial cells and the low-dose stimulatory response following overcompensating for this, which is namely hormesis. The abnormal development of sea-urchin larvae was significantly different from control after exposure to sediments from P1, P2, P3 and P4. Regarding *I. galbana* and *T. chuii* microalgae's growth, it was observed a significant increase after exposure to sediments from P1, P2 and P4 ( $p < 0.05$ ) which showed the possible eutrophication process in areas affected by wastewater discharges. Sediment affected by WWTP in Puerto Real and El Puerto de Santa María cities was considered the most toxic ones between the studied areas. The results of different species and sediment phases found in this study highlight the importance of the employment of a multi-trophic battery of bioassays with different sediment phases and exposure-times in order to assess environmental quality of sediments affected by wastewater discharges.

**TP252 Metabolic fingerprinting and behavioral responses as indicators of contaminant exposure in copepods** B. Hansen, SINTEF Materials & Chemistry / Marine Environmental Technology; I. Fines, C. Miljeteig, Norwegian University of Science and Technology; D. Altin, BioTrix; T. Nordtug, SINTEF Materials and Chemistry / Marine Environmental Technology;



A.J. Olsen, Norwegian University of Science and Technology; T.R. Storseth, SINTEF Fisheries and Aquaculture. Untargeted metabolic fingerprinting can be used in order to assess global changes in the metabolome, to determine effect levels and to assess recovery of molecular homeostasis following exposure. However, such changes should be put into an ecologically relevant context. In copepods, alterations in normal behavioral caused by exposure to contaminants may have severe implications for survival as they rely on escape responses to avoid being preyed by fish. Thus, behavioral changes may be used as an ecologically relevant endpoint. In the present experiment copepods (*Calanus finmarchicus*) were exposed to sublethal concentrations of water soluble fractions (WSF) of North Sea crude oil (24-96 hrs). Every 24 hrs, copepods were subjected to a physical stress (hydrodynamic disturbance) and behavioral parameters were measured (escape response frequencies, escape response latency time and fatiguing effects of repetitive stress). Simultaneously, copepods were sampled for metabolic fingerprinting using <sup>1</sup>H-nuclear magnetic resonance (<sup>1</sup>H-NMR). <sup>1</sup>H-NMR metabolic fingerprints were recorded on a Bruker Avance DRX 500 NMR spectrometer, and the data imported to Matlab where Principal Component Analysis (PCA) and Partial Least Squares (PLS) regression was used to study differences in the metabolic fingerprints between the groups. Similar development of response patterns were observed during exposure over time for behavioral and metabolic fingerprints. The results indicate that behavioral alterations and metabolic fingerprinting may both be used as sensitive indicators of exposure for copepods exposed to crude oil WSE.

**TP254 Response of fathead minnow and *Ceriodaphnia dubia* to pulp and paper mill effluent exposure: pattern changes associated with mill process upgrades** C. Flinders, W. Streblow, R. Philbeck, D. Borton, D. Cook, NCASI. Although pulp & paper mill effluents have sometimes been implicated in biological responses, process upgrades such as effluent biotreatment & best management practices (BMP) to reduce liquor losses have sometimes corresponded to reduced responses. Despite this, studies documenting changes in biological response to effluent exposure with mill process upgrades are rare. We conducted 7-d fathead minnow & *Ceriodaphnia dubia* bioassays (n=2-3) in conjunction with each of four fathead minnow life cycle tests over a 10-y period to evaluate changes in biological response to combined bleached/unbleached kraft mill effluent exposure as mill process upgrades were implemented. Life cycle & associated tests were conducted prior to, during (n=2), & following the completion of process upgrades. Upgrades included 100% ClO<sub>2</sub> bleaching, condensate stripping, reduced liquor losses, conversion to aerobic secondary treatment, improved BMPs, O<sub>2</sub> delignification, & reduced water use. Following upgrade completion, treated mill effluent had reduced concentrations of BOD (56%), COD (39%), color (36%), & polyphenols (66%) relative to pre-upgrade effluent, while phytosterols & resin-, chlorinated resin-, & fatty acids were reduced by ≥95%. Biological response to effluent exposure was reduced following the completion of process upgrades relative to pre-upgrade effluent. The IC<sub>25</sub> for *Ceriodaphnia* reproduction prior to mill upgrades was 12%, & increased to 31% following upgrade completion. No effluent-related response was seen in fathead minnow 7-d survival & growth in any test during the study. Prior to mill upgrades, 28- & 56-day survival & growth of juvenile fathead minnow were significantly lower in higher effluent concentrations than controls (IC<sub>25</sub>=48-98%), but showed no effluent-related differences following completion of mill upgrades. Egg production with exposure to post-upgrade effluent increased (IC<sub>25</sub>=58% effluent) compared to pre-upgrade effluent (IC<sub>25</sub>=23%). Other measured endpoints (female liver somatic index (LSI), male testosterone & 11-ketotestosterone, & gonad histology) showed improvement following process upgrades while others showed inconsistent effluent-related patterns (egg hatchability & diameter, gonad somatic index, male LSI, vitellogenin, & numbers of tubercles). Overall, changes in effluent quality associated with mill process upgrades appear to be associated with improved biological responses to treated effluent at this mill.

**TP255 Sex Ratios and Body Size of Gulf Killifish (*Fundulus grandis*) From Sites in Barataria Bay, LA. Impacted by the Deepwater Horizon Oil Spill** J. Carr, Texas Tech University / Biological Sciences Department; D.L. Carr, Texas Tech University / Biological Sciences; E. Smith, Texas Tech University; A. Thiyagarajah, Tulane University. The long-term goal of this research is to assess the impact of contaminants from the Deep Water Horizon oil spill on the reproductive status of the Gulf killifish (*Fundulus grandis*). We sampled 11 sites throughout Barataria Bay and just west of the Mississippi Delta for *F. grandis* during August-September 2011. *F. grandis*

was found at two impacted sites in Barataria Bay and both reference sites in East Texas. The greatest number of *F. grandis* were collected at the reference sites. At the Sabine Pass reference site we collected 220 *F. grandis* while at McFaddin National Wildlife refuge (NWR) we collected eight *F. grandis*. At two sites in Barataria Bay that were sampled in all three months, Bay Jimmy (most impacted) and Bayou St. Denis (less impacted) we trapped 14 and 46 *F. grandis*, respectively. Body weights of *F. grandis* from the Sabine Pass reference site ranged from 1.06 g to 31.8 g. Equally large *F. grandis* were collected from McFaddin NWR. *F. grandis* collected from Bay Jimmy ranged from 1.25 g to 7.96 g. *F. grandis* collected from Bayou St. Denis ranged from 0.58 g to 11.2 g. Thus, we never collected sexually mature *F. grandis* greater than 12 g from any of the sites in Barataria Bay. There were no differences in body weights between Sabine Pass, Bay Jimmy, or Bay St. Denis over three sampling trips. However, mean body weights of *F. grandis* from McFaddin NWR were statistically greater than any of the other three sites analyzed (p < 0.05). Sex of all fish preserved in formalin was determined using secondary sex characteristics and visual inspection of gonadal phenotype. At Sabine Pass there were 100 females and 91 males for a sex ratio of 1:0.91 F:M. At site Bayou St. Denis the sex ratio was 6 females to 26 males or 1:4.3 F:M. For Bay Jimmy, the sex ratio was 3 females to 8 males or a ratio of 1:2.7 F:M. At McFaddin NWR, seven of eight *F. grandis* collected were female. Thus, sex ratios were skewed towards fewer females in Barataria Bay. In conclusion, there were fewer animals at the large end of the body weight range collected from Barataria Bay. Whether these differences in number of *F. grandis* reflect the impact of the DWH oil spill is unknown and awaits contaminant analysis in tissues and water samples. This work was supported by a grant from the Gulf of Mexico Research Initiative.

**TP256 Spatial and temporal variability of biochemical responses in resident fish after the M/V Hebei Spirit oil spill (Taean, Korea)** J. Jung, M. Kim, U. Yim, Y. Che, H. Kim, S. Ha, J. An, G. Han, W. Shim, Oil and POPs Research Group, Korea Ocean Research and Development Institute. After the Hebei Spirit oil spill incident (7th December, 2007) in the west coast of Korea, biliary PAH metabolite and hepatic biomarkers in a pelagic and a benthic fish was monitored for four years. Concentrations of 16 PAHs and alkylated PAHs in fish muscle were highest (22.0 ng/g d.w. for 16 PAHs and 284 ng/g d.w. for alkylated PAHs) at 5 days after the spill and then decreased rapidly to background levels at 11 months after the spill. Fish from the oiled site had elevated biliary PAH metabolite concentrations immediately after the spill; these declined steadily in both species, but were still above reference site value. Regarding our results of detoxification response, markers of Phase I followed a similar trend with levels of biliary metabolites, while markers of Phase II and GST appeared relatively unchanged. CYP1A activity (induction/inhibition) was fluctuated in both species. Brain acetylcholinesterase activity was not related to oil exposure. The high correlation rate between the CYP 1A hepatic detoxification system and PAHs degradation as shown by biliary PAH metabolites in both species further supports the use of CYP 1A as a reliable biomarker of oil exposure in fish, especially when both measurements are used complementarily.

**TP258 The effects of short-term exposure to urban surface runoff on reproductive, immune, and oxidative stress biomarkers in the fathead minnow** C. Miresse, E. Smith, R. Goldenstein, University of Saint Thomas; H. Schoenfuss, St. Cloud State University / Aquatic Toxicology Laboratory, St. Cloud State University / Department of Biological Sciences WSB-273, St. Cloud State University / Department of Biological Sciences MS-273; T. Minarik, MWRDGC / Associate Aquatic Biologist; D. Martinovic, University of Saint Thomas, University of St. Thomas / Dept. of Biology, U.S. EPA, Mid-Continent Ecology Division / Mid-Continent Ecology Division, University of St. Thomas / Biology, University of Saint Thomas / Biology. Many studies have documented the occurrence of environmental estrogens (EEs) in wastewater treatment plant effluents (WWTPes), but urban aquatic systems may also be impacted by EEs and other micropollutants contained in surface runoff. Furthermore, untreated wastewater can be released into surface waters as combined sewer overflows (CSOs) during periods of heavy rain or rapid snow melt. The present study was conducted within the Metropolitan Water Reclamation District of Greater Chicago (MWRDGC), which receives pollutant loads from seven WWTPes and many gravity CSOs. The primary objective of the study was to determine whether surface runoff and CSOs may be contributors of EEs to the MWRDGC watersheds. We conducted 48h exposures of adult male fathead minnows (n=8 per treatment) to control water, surface runoff samples collected from



six locations, and to one CSO sample. RNA was extracted from the liver, and gene expression analyzed using quantitative real time polymerase chain reaction. Vitellogenin and estrogen receptor mRNA increases were used as indicators of EEs. In addition, we examined whether runoff has the potential to impact expression of genes involved in reproduction (steroidogenic acute regulatory protein, 3- $\beta$ -hydroxysteroid dehydrogenase, 11- $\beta$ -hydroxysteroid dehydrogenase), immune functioning (interleukins 6, 12), and oxidative and/or metabolic stress responses (glutathione reductase, glutathione S-transferase alpha, metallothionein). It was shown that expression of several genes (e.g., vitellogenin, metallothionein, interleukins) relative to control groups, was increased upon exposure to water samples from multiple run-off sites (including CSO), though responses were highly variable likely due to short exposure times and variability in the sensitivity of individuals. Our results indicate that surface runoff and CSOs may be important contributors of EEs and other contaminants in urban ecosystems.

**TP259 The effects of varying environmental conditions on the toxicity of mine effluent constituents to coldwater fish** D. Moore, University of Guelph / School of Environmental Sciences; D.G. Poirier, Ontario Ministry of the Environment / Laboratory Services Branch; P. Sibley, University of Guelph / School of Environmental Sciences, University of Guelph / Department of Environmental Biology; K. Solomon, University of Guelph / School of Environmental Sciences, University of Guelph / Centre for Toxicology, School of Environmental Sciences. Due to method standardization, current regulations may not afford protection against adverse effects of industrial developments under non-standard pH, temperature, and hardness conditions of arctic waters. These effects may be due to liberated minerals, processing by-products, and service community wastes. Canadian mines are controlled under the Fisheries Act, regulating "Any substance that is...deleterious to fish or fish habitat or to the use by man of fish that frequent that water". These regulations limit effluent parameters based on toxicological evidence produced via standard assay methods published by Environment Canada. For coldwater fish, rainbow trout is used as a surrogate and employs conditions optimal to that species. In contrast, locations of concern exhibit temperatures 10°C colder than standard test conditions, hardness 2-10 times lower than dechlorinated water, and variable pH due to contamination and natural geology. The study aims to characterize the variability of effluent toxicity across a range of species and conditions while assessing whether the current standard test is protective enough to cover this variation by comparison of standard and 'amended standard' (varied conditions) assays. Seventeen contaminants (NaCl, NaNO<sub>2</sub>, NaNO<sub>3</sub>, Na<sub>2</sub>SO<sub>4</sub>, KCl, CaCl<sub>2</sub>, Cd, Cr, As, Cu, Zn, Mg, Ni, Al, MoO<sub>4</sub>, Se, NH<sub>4</sub>OH,) were tested using five species of fish native to Northern Canada (arctic charr, lake trout, lake whitefish, round whitefish, and arctic grayling) across a range of pH (5.5, 6, 6.5, 7, 7.5, and 8), temperature (5, 10, and 15°C), and hardness (30, 60, and 120ppm) conditions and their subsequent observed effects modeled as 24, 48, 72, and 96-hour LC50 values. By comparing these results to each other and to those of the standard rainbow trout test, we have assessed the effect of pH, temperature, and water hardness on contaminant toxicity and time-to-toxic effect within and between species while characterizing the ecological protection afforded by current regulations. The data suggests that, in some instances, the standard has failed to capture the potential toxicity exhibited in non-standard species and non-standard, real-world conditions. Should this variability of non-standard conditions exceed the resiliency of the standard test to act as a surrogate, the mandate of the Fisheries Act to provide adequate protection to fish, fish habitat, and human use of fish will not be met.

**TP260 Toxicity Identification Evaluation of WWTP Effluent Case Study: Dissolved Carbon Dioxide as the Primary Toxicant** Y. Yang, East Bay Municipal Utility District; D. Jackson, East Bay Municipal Utility District / Laboratory. Whole effluent toxicity testing is commonly required for wastewater treatment plants (WWTP) in the US for assessing potential toxicity. Detection of toxicity usually leads to a Toxicity Reduction Evaluation / Toxicity Identification Evaluation (TRE/TIE) to determine the cause of the toxicity. The East Bay Municipal Utility District (EBMUD) conducts monthly acute flow-through tests using larval rainbow trout on the discharge from its treatment plant in Oakland, California. Starting in 2010, EBMUD observed significant acute toxicity for an extended period. The test fish mortality was invariably preceded by distinct stress signs, occurring minutes after exposure to plant effluent. With all effluent limits being met, an unknown toxicant appeared to be present. To identify the cause of toxicity, EBMUD conducted a systematic three-phase TIE according

to US Environmental Protection Agency's guidelines. Results from TIE Phase I suggested existence of a volatile toxicant that had increased toxicity at lower pH. These characteristics were employed to develop a closed-loop aeration system that allowed successful recovery of the toxicant. A series of tests and literature search were then conducted, leading to a preliminary identification of dissolved CO<sub>2</sub> as the toxicant. Subsequent tests in Phase III confirmed this hypothesis. Static tests gave qualitative evidence for a direct link between dissolved CO<sub>2</sub> level and test fish stress behavior. Continuous flow-through tests demonstrated a reduction of toxicity after removing dissolved CO<sub>2</sub> and a complete restoration of toxicity upon reintroduction of the compound, confirming that the primary toxicant causing the unusual fish mortality in this effluent was dissolved CO<sub>2</sub>. In summary, the TIE investigation at EBMUD concluded with successful toxicity identification. The findings, approaches, and lessons learned from this investigation are applicable to other wastewater treatment plants, especially high purity oxygen activated sludge treatment plants where high-alkalinity and low-pH effluents may produce conditions resulting in a similar toxicity profile.

**TP261 Toxicity of Coal Fly Ash Leachate to Luminescent Bacterium *Vibrio fischeri*** S. Ikeda, Tokai University / Graduate School of Earth and Environmental Sciences; I. Kostova, Sofia University / Faculty of Geology and Geography; H. Sekine, Tokai University / Graduate School of Science; Y. Sekine, Tokai University School of Science / Department of Chemistry. Coal combustion supplies more than 48% of the electricity generated in Bulgaria and 25% in Japan. Coal fly ash is a residue of coal-fired thermoelectric power plants (TPPs). It is known for containing various toxic elements. Coal fly ash is recently applied as filler of asphalt or cement and used for construction. Numerous papers described its mutagenicity and genotoxicity. However, acute chemical toxicity to luminescent bacterium *Vibrio fischeri* has not been evaluated. This study aimed to provide new insights about the influence of coal combustion products on bacterial activity. It is achieved by characterizing the bioluminescence inhibition property of coal fly ash collected from TPPs, bioassay using luminescent bacterium *Vibrio fischeri* and chemical analyses. Fly ash samples were extracted with 10 mL of sterilized distilled water by ultrasonic extraction and filtrated by 0.45  $\mu$ m  $\Phi$  membrane filter. Then the filtrates were mixed into bacterial solution. The bioluminescence intensity of water solutions was measured by a luminometer. Heavy metallic components were also separated from coal fly ash samples by extraction with 10 mL of 1M-hydrochloric acid. The time courses of bioluminescence intensity for acid extracted solution were also measured. Chemical components of sample solutions were determined by chemical analyses. As a result, water extractions of the fly ash samples significantly inhibited the bacterial activity, in the cases when the pH of the extractions were greater than 9 or smaller than 6. pHs of aqueous solution of fly ash from bituminous coal were close to neutral pH. Fly ash by the combustion of lignite caused relatively greater pH (9.1-11.8) of water leachates. The pH seemed to relate to sourcing area of coals and/or coal rank. On the other hand, the pH values of water leachates ranged from 6 to 9 showed activation effect on the bacteria. This means acidic or basic property of coal fly ash leachates determines its toxicity. Heavy metals in fly ash also inhibited the luminescence of *Vibrio fischeri* remarkably. No sample activated the bacterial luminescence. This study showed that bacterial test is a possible tool for detecting hazardous property of the complex effluents.

**TP262 Toxicity of sediment dilutions of East Branch Grand Calumet River to *Hyalella azteca*, *Chironomus dilutus* and *Lampsilis siliquoidea*** J.L. Kunz, USGS, US Geological Survey / Department of Toxicology; C.G. Ingersoll, USGS / Columbia Environmental Research Center, U.S. Geological Survey / Columbia Environmental Research Center; J.P. Hughes, USGS; S. Ireland, U.S. EPA / Great Lakes National Program Office; D.R. Mount, US Environmental Protection Agency / ORD; T.W. Valenti, Syngenta Crop Protection LLC / National Research Council, National Research Council / U.S. Environmental Protection Agency; J. Hockett, USEPA Environmental Effects Research Laboratory. Sediments at sites within the East Branch of the Grand Calumet River (EBGCR; located south of Lake Michigan) exhibit elevated concentrations of metals, PAHs, PCBs, and oil and grease. The objective this study was to evaluate the toxicity of two dilution series of an EBGCR sediment. Test organisms included amphipods (*Hyalella azteca*; 28-d exposures measuring effects on survival, weight, or biomass), midge (*Chironomus dilutus*; started with < 1-h-old larvae or with 7-d-old larvae measuring effects on survival, weight, biomass, or emergence), and mussels (*Lampsilis siliquoidea*; 28-d exposures measuring effects on survival,

weight, or biomass). One dilution series (0%, 6.25%, 12.5%, 25%, 50%) was prepared using sand as the diluent low TOC and a second dilution series was prepared using West Bearskin (WB) sediment as the diluent (high TOC [about 10%], similar to the TOC in the SS-05 sediment). All three test organisms were more sensitive with exposures to low TOC dilutions compared to high TOC dilutions. Effects in longer-term exposures were generally more pronounced compared to shorter-term exposures. Midge in exposures started with < 1-h-old larvae were more sensitive than amphipods or mussels or more sensitive than midge in exposures started with 7-d-old larvae, but replicate variability was higher in exposures started with < 1-h-old larvae. Exposures to low TOC dilutions reduced survival and biomass of amphipods and midge at every tested concentration (as low as 6.25%), whereas exposure to high TOC dilutions showed effects only at higher concentrations. In particular, the low TOC dilutions caused complete mortality of < 1-d-old midge at concentrations as low as 6.25%. Mussel survival, weight and biomass were reduced at every tested concentration in the sand dilution, but these reductions were less pronounced than those in amphipod or midge exposures. Exposure to high TOC dilutions reduced mussel biomass in a dose-dependent fashion, but not mussel survival. Overall, the protective effect of the higher-TOC diluent is consistent with the hypothesis that the toxicity of the sediment is likely driven by PAHs, rather than oil and grease. In addition to using laboratory manipulations of a field-collected sediment to better understand relative endpoint sensitivity of test organisms, this study also advances methods for toxicity testing with < 1-d-old midge.

**TP263 Tracing toxicants in Hantan river watershed using toxicity identification evaluation methods** J. Yoo, Korea University / Division of Environmental Science & Ecological Engineering; J. Jung, Korea University / Div. of Environmental Science & Ecological Engineering, Korea University / Associate Professor; T. Han, University of Incheon / Department of Life Sciences. Toxicity-based regulations of water quality have been adopted to complement the conventional discharge limits based on chemical analyses. In this study, a long-term toxicity monitoring was conducted from May to October 2011 in Hantan river watersheds using *Ulva pertusa* and *Daphnia magna*. Persistent acute toxicity to both species was observed at a Hanbawi gyo site, leading to identification of the source of acute toxicity. Among point sources, discharges from a dyeing industrial complex factory were attributable to the toxicity of downstream water ( $r^2 = 0.8044$  for *U. pertusa*). In addition, toxic substances in the industrial effluent were identified as Cu and Zn, and their concentrations were well correlated to those in downstream water at the Hanbawi gyo site ( $r^2 = 0.8665$  and  $0.9634$ , respectively). These findings suggest that Cu and Zn in effluent from the industrial complex may induce acute toxicity in a receiving water body, Pocheon stream.

**TP264 A Methodological Proposal to Identify Terrestrial Sensitive Areas to Hydrocarbon Spills** A. Mendoza-Cantu, Instituto Nacional de Ecología / Dirección General de Investigación sobre la Contaminación Urbana y Regional; S. Cram, Universidad Nacional Autónoma de México / Instituto de Geografía; L. Cedillo, Instituto Nacional de Ecología / DGICUR. Mexican oil industry holds a broad pipeline net that is extended along the whole country. This net crosses many important urban, industrial, agricultural and natural areas, which can be affected by hydrocarbon leaks and spills. These events can be a threat for natural resources, ecosystems, human population and economic activities. In this context, the National Institute of Ecology, in collaboration with the Geography Institute of the Mexican National Autonomous University, has carried out a research project directed to develop a methodology to identify environmental sensitive areas to hydrocarbon spills. As the first phase of this project, the methodological proposal was developed as a pilot study in Coatzacoalcos region, in the state of Veracruz. This proposal sets out to assess the environmental sensitivity as a function that relates a threat with vulnerability. In this case, the threat was defined as an oil spill from a pipeline, and the vulnerability was described considering all the intrinsic characteristics that make a zone susceptible to the adverse effect of an oil spill. The studied region was divided and classified into many basic functional units, called geosystems. The vulnerability of these units was evaluated in five different levels considering the expected behavior of oil spills in terrestrial zones and flooding zones. These geosystems were ranked taking into account its physical, biological, and socioeconomic characteristics. As the main product of this study an environmental sensitivity map was built using a geographic information system. All the geosystems within

Coatzacoalcos region with different vulnerability level can be located in this map.

**TP265 An Investigation into the Effectiveness of Municipal Drinking Water Treatment Processes to Remove EDCs and PPCPs** K. Auger, K.F. Arcaro, UMass Amherst / Veterinary and Animal Sciences; D. Reckhow, UMass Amherst / Civil and Environmental Engineering. The objective of this study was to investigate the effectiveness of water treatment processes to remove selected endocrine disrupting compounds (EDCs), pharmaceuticals and personal care products (PPCPs), and their daughter products from municipal drinking water supplies. Both chemical analyses and biological assays were used to assess the presence of EDCs and PPCPs. Here we present results from two biological assays that are highly sensitive to the presence of estrogenic and dioxin-like compounds in water: induction of vitellogenin (VTG) mRNA and induction CYP1A1 in the livers of male fish. Source water samples were collected from each of four drinking water utilities in the northeast. A portion of the Source water sample from each utility was amended with a "cocktail" of EDCs and PPCPs, which included some estrogenic and CYP1A1-inducing compounds, to produce a Spiked water sample. A portion of this amended water sample was run through a bench scale simulation of the water treatment process used at the drinking water utility to provide a Finished water sample. To determine the presence of estrogenic and CYP1A1-inducing activity in the Source water and the ability of the drinking water utility treatment process to remove the activities induced by the cocktail, male Japanese medaka fish were exposed to Source, Spiked, and Finished water for 72 hours. Hepatic VTG mRNA levels were measured in males from all exposures as well as positive and negative controls. None of the Source water samples induced estrogenic or dioxin-like activity. As expected, the Spiked water samples significantly induced both VTG and CYP1A1. The estrogenic activity was eliminated by three of the four treatment processes as fish exposed to three of the Finished water samples did not show an increase in VTG. Similar results were obtained for CYP1A1. In summary, our data demonstrate that none of the source waters used by the four drinking water utilities contained compounds that were able to significantly induce VTG or CYP1A1 in a sensitive fish model. Furthermore, if such compounds were present, the treatment processes currently in use at three of the four utilities would effectively remove the compounds while the treatment process of one utility would not.

**TP266 Evaluating the Toxicity of Hypersaline Brine Using Nine California Ocean Plan Toxicity Test Protocols** L.L. Jennings, University of California, Davis-Granite Canyon Marine Pollution Studies Laboratory / Environmental Toxicology; B. Phillips, University of California, Davis-Granite Canyon Marine Pollution Studies Laboratory / Environmental Toxicology; B. Anderson, University of California, Davis-Granite Canyon Marine Pollution Studies Laboratory / Environmental Toxicology; J. Voorhees, University of California, Davis-Granite Canyon Marine Pollution Studies Laboratory / Environmental Toxicology; S. Katz, K. Siegler, R. Tjeerdema, University of California, Davis-Granite Canyon Marine Pollution Studies Laboratory / Environmental Toxicology; J. Jensen, California State Water Resources Control Board. As water needs increase in California, coastal cities are exploring ocean desalination as a freshwater supply alternative. Desalination results in the discharge of hypersaline brine to the ocean, and there is concern this discharge could impact marine receiving waters. This study determined the salinity tolerance of seven marine organisms using nine California Ocean Plan protocols. Test organisms included: red abalone (*Haliotis rufescens*), giant kelp (*Macrocystis pyrifera*), bay mussel (*Mytilus galloprovincialis*), mysid shrimp (*Americamysis bahia*), topmelt (*Atherinops affinis*), purple sea urchin (*Strongylocentrotus purpuratus*) and sand dollar (*Dendraster excentricus*). Salinity tolerances were determined with an initial range-finder test followed by two definitive tests. Results showed that salinity tolerance varied by protocol. Euryhaline species were more tolerant to higher salinities than were marine species. The most sensitive organisms, endpoints, and their median effects salinities were: sea urchin, sand dollar and abalone development (38‰) > sand dollar fertilization (41‰) > mussel development (43‰) > sea urchin fertilization (44‰) > mysid survival (48‰) > kelp germination (56‰) > topmelt survival (62‰). Results of these experiments will be used by the State Water Resources Control Board to establish discharge requirements for desalination facilities.



**TP267 The Use of Toxicity Identification Evaluation (TIE) Procedure to Investigate the Toxicity of ArcelorMittal Tubarao Effluents** E. Passamani, ArcelorMittal Tubarao / Environment; T. Furley, APLYSIA Environmental Research and Solutions; J.O. Santos, ArcelorMittal Tubarao / Environment. ArcelorMittal Tubarao, the main world supplier of steel slabs, is located 14km from Vitoria, the capital of the State of Espírito Santo, in Brazil. The company's domestic and industrial effluents, previously treated, are mixed with the sea water used to cool equipment and structures and, after that, thrown into the marine environment. Since 2008, the company has been performing eco-toxicological tests using test organisms such as *Vibrio fischeri*, *Skeletonema costatum* and *Echinometra lucunter* on its final effluents in order to evaluate the toxic effect of this effluent on the receiving marine environment. The present work aims to apply the TIE procedure to investigate the ecotoxicity of ArcelorMittal Tubarao effluents using sea urchins – *Echinometra lucunter* – as test organism. The study was carried out in two steps: the first step consisted in the ecotoxicological evaluation of seven sectorial effluents and the final effluent from simple and composed samples and it was verified that three sectorial effluents presented toxicity; while the second step consisted in applying the TIE procedure to the three most important sectorial effluents identified in the first step and the final effluent. The Sectorial effluent RB22 lost its toxicity after the elimination of ammonia. For the sectorial effluent RB23 toxicity may be connected to a series of mixed compounds such as metals, ammonia or any other nitrogen based substance and nonpolar organic compounds, and the removal of one of them does not ensure the elimination of the effluent ecotoxicity. The sectorial effluent ETB (coke plant wastewater treatment) toxicity was caused by the presence of ammonia or other nitrogen based substance followed by organic compounds and cyanide. The toxicity of the final effluent was (mostly) caused by nonpolar organic compounds and nitrogen compounds.

**TP268 Alkaline hydrolysis of explosives-contaminated soils from shooting and artillery ranges in South Korea** S. Oh, University of Ulsan / Department of Civil and Environmental Engineering, University of Ulsan / Associate Professor; D. Shin, M. Yoon, University of Ulsan. In this study, we investigated the kinetics and pathways of alkaline hydrolysis of nitro explosives in contaminated soils sampled from shooting and artillery ranges in South Korea. It was hypothesized that alkaline hydrolysis may effectively transform the nitro explosives in soils. The concentrations of TNT and RDX in contaminated soils were 5.5 and 25.6 mg/kg, respectively. In homogeneous aqueous systems, 2,4-dinitrotoluene (DNT), 2,4,6-trinitrotoluene (TNT), and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) were rapidly removed in solution at pH  $\geq 12$ . Estimated pseudo-first-order rate constants for the alkaline hydrolysis of DNT, TNT, and RDX at pH 13 were 0.095, 2.528, and 0.019 min<sup>-1</sup>, respectively. In heterogeneous system with explosives-contaminated soils, the degradation of explosives via alkaline hydrolysis was also greatly enhanced at pH  $\geq 12$ . When a soil-to-water ratio was more than 1/6, the extent of alkaline hydrolysis was limited though the solution pH was 12-13. The results indicated that the alkaline hydrolysis of explosives in the heterogeneous system was strongly affected by the soil-to-solution ratio. Product identification by GC-MS analysis showed that DNT and TNT were hydrolyzed via leaving of methyl function, ring cleavage, and polymerization. Biodegradability and toxicity of hydrolysis products are under investigation.

**TP269 Analysis of BTEX groundwater concentration from surface spills associated with hydraulic fracturing operations** S. Gross, B. Tvermos, H. Avens, A. Banducci, J. Panko, J. Sahmel, ChemRisk. Concerns have arisen among the public regarding the potential for drinking water contamination from the migration of methane gas and hazardous chemicals associated with hydraulic fracturing and horizontal drilling. However, little attention has been paid to the potential for groundwater contamination resulting from surface spills at active well sites. Recent articles published in the Colorado news media suggested that surface spills at well sites located in Weld County, Colorado were associated with the release of benzene at concentrations markedly exceeding state standards. In 2011, Weld County, Colorado was home to nearly 18,000 active wells producing both gas and oil. Wells producing crude oil in addition to methane gas are a potential source of petroleum hydrocarbon release into groundwater via surface spills. Of particular interest is release of benzene, toluene, ethylbenzene, and xylene (BTEX) which are present in low percentages in crude oil, and at sufficient doses, have been associated with adverse human health effects. To explore the impact of surface spills on groundwater resources, we analyzed data from

77 spills with groundwater impact in Weld County reported by operators to the Colorado Oil and Gas Conservation Commission (COGCC) from July 2010 to July 2011. Less than 0.5% of the active wells experienced a surface spill impacting groundwater during the study period. Groundwater samples were analyzed for BTEX per COGCC protocol. For samples taken both within the spill excavation area and on the first reported day of sampling, the BTEX measurements exceeded National Drinking Water maximum contaminant levels (MCLs) in 90, 30, 12, and 8% of the samples, respectively. However, actions taken to remediate the spills were effective at reducing BTEX levels, with at least 84% of the spills resolved as of May 2012. In this study, we offer recommendation for best practices to companies drilling in Weld County as well as recommendations for the necessity of community outreach programs by the COGCC regulatory agency.

**TP270 Analysis of Polychlorinated Biphenyls (PCBs) congeners in fish from Savannah River, Georgia** L. Hao, Savannah State University / Department of Natural Science; K. Kumar, M. Gilligan, P. Li, R. Johnson, Savannah State University; B. Loganathan, Murray State University; K. Sajwan, Savannah State University. The purpose of the present study was to determine the contamination profiles of 17 Polychlorinated Biphenyls (PCBs) congeners in fish from Savannah River, Savannah, Georgia, USA. Four fish species, including Whiting, Southern flounder, Spot, and Weakfish, were collected in the Savannah River in September, 2008. The  $\Sigma$ PCBs concentration of Southern flounder was the highest (983.7 ng/g lipid wt). Weak fish had the lowest concentration (286.4 ng/g lipid wt). There was no significant difference among the four species ( $p=0.0041$ ). PCB-1 was the predominant congener for all the fish species except spot, for which PCB 52 was the predominant pesticide which contributed 27% to the  $\Sigma$ PCBs concentration. The levels of in fish Savannah River were below the FDA established limits for human consumption.

**TP271 Changes in Dissolved Phase PCBs and Hexachlorobenzene (HCB) Concentrations in the Ashtabula River Pre- and Post-Environmental Dredging** J. Banda, D. De Vault, K. Tloczynski, U.S. Fish and Wildlife Service. The Ashtabula River, an area of concern in the Great Lakes, was severely impacted by PCBs, HCB and other chlorinated industrial compounds primarily originating from approximately 1.6 miles upstream of Lake Erie at the Fields Brook Superfund site. Following cleanup activities at Fields Brook, environmental dredging was conducted on the Ashtabula River between 2006 and 2008. High volume water samples were collected in June and August 2001 (pre-dredging) and in June and August 2011 (post-dredging). In June, mean concentrations of dissolved phase total PCBs declined from 44.6 ng/l in 2001 to 3.4 ng/l post-dredging and mean dissolved phase HCB concentrations declined from 11 ng/l to 0.6 ng/l. Significant but lesser declines in mean dissolved phase total PCB and HCB concentrations were observed between samples taken in August 2001 and August 2011. Dissolved phase total PCB and HCB concentrations were highest near the confluence of Fields Brook and declined downstream toward Lake Erie in 2001. In 2011 concentrations increased above background at river mile 1.6 with no spatial pattern observed downstream. These data indicate that remedial actions have reduced PCB and HCB loadings from Fields Brook. However, concentrations still exceed water quality criteria and objectives for protection of human health and wildlife species. Fish samples were also collected and analyzed in 2001 and 2011. PCB and HCB concentrations in both fish and water following remedial actions will be discussed.

**TP272 Trends of Persistent Global Contaminants in the Flesh of Farmed Salmon, 2003-2010** J.P. Benskin, AXYS Analytical Services Ltd; M.G. Ikononou, Institute of Ocean Sciences; K. Ang, Stolt Sea Farm Inc.; E.N. Friesen, Skretting Canada Inc.; D.A. Higgs, DFO / University of British Columbia / Centre for Aquaculture and Environmental Research; C. Dubetz, Institute of Ocean Sciences. Salmon are acknowledged to be among the best dietary sources of omega-3 (n-3) highly unsaturated fatty acids (n-3 HUFAs). Omega-3 fatty acids are essential for normal metabolism and have been linked with a number of positive health effects (e.g. cognitive development and reduced risk of some types of cancer). Following increased public concern over contaminant concentrations in farmed salmon in 2004, a concerted effort was made by the Canadian aquaculture industry to reduce contaminant loadings in farmed fish. In the present work, the impact of modifying feed formulations on the flesh quality of farmed salmon was investigated from 2003-2010. Concentrations of polychlorinated biphenyl



compounds (PCBs), pesticides, polychlorinated dibenzodioxins/dibenzofurans (PCDDs/PCDFs), and polybrominated diphenyl ethers (PBDEs), along with a variety of fatty acids were measured in commercial feed (2003-2010,  $n=50$ ) and skinned fillets of farmed Atlantic, Coho, and Chinook salmon collected from farms around coastal British Columbia (B.C.; 2003-2007;  $n=226$ ) and New Brunswick (N.B.; 2007-2010;  $n=163$ ). Wild Coho, Chinook, Chum, Sockeye, and Pink salmon from B.C. were also examined (2003-2007,  $n=223$ ). Overall, a significant decline was observed for PCB and pesticide concentrations (wet weight basis) in farmed salmon from 2003-2010, consistent with observations in feed. In fact, PCB and pesticide concentrations in farmed salmon collected from N.B. in 2010 were similar to, and in some cases lower than concentrations observed in B.C. wild salmon between 2003 and 2007. PCDD/PCDF and PBDE concentrations were not observed to decrease substantially from 2003-2010 (consistent with feed) but were nevertheless orders of magnitude lower than other foods and similar to concentrations in wild salmon. These data indicate that changes in feed formulations over the last decade have decreased overall contaminant concentrations in farmed salmon to levels consistent with wild salmon.

**TP273 New Standard Reference Materials (SRMs) for Environmental Contaminant Analysis** J. Hogue, National Institute of Standards and Technology (NIST) / Analytical Chemistry Division; M. Schantz, National Institute of Standards and Technology / Analytical Chemistry Division; J. Kucklick, J.M. Keller, S. VanderPol, R. Pugh, A. Moors, S. Wise, National Institute of Standards and Technology. For the past 30 years, the National Institute of Standards and Technology (NIST) has developed Standard Reference Materials (SRMs) in response to a growing demand for natural environmental matrices with certified values for organic contaminants. The first environmental SRM issued in 1980, SRM 1580 Organics in Shale Oil, had certified concentrations for five polycyclic aromatic hydrocarbons (PAHs), three phenols, and benzo[*a*]quinoline. Since this time, the demand for more environmental matrix SRMs has increased. This has led NIST to produce a larger variety of environmental matrix SRMs including whale blubber, fish and mussel tissues, human blood, house dust, sewage sludge, air particulate material, and a variety of sediments, with a range of individual organic contaminants characterized to better suit the needs of the environmental community. Regarding the marine environmental community, NIST has issued several new SRMs, including SRM 2974a Organics in Freeze-Dried Mussel Tissue (*Mytilus edulis*) and SRM 1588c Organics in Fish Oil. NIST has also replaced SRM 1941a Organics in Marine Sediment with SRM 1941b and has recently certified SRM 1974c Organics in Mussel Tissue (*Mytilus edulis*), to replace SRM 1974b. These SRMs have been characterized for a suite of organic contaminants, including polychlorinated biphenyl (PCB) congeners, PAHs, polybrominated diphenyl ether (PBDE) congeners, and organochlorine pesticides. This presentation will summarize the development and certification of these SRMs.

**TP274 PCB Congeners, Chlorinated Pesticides and PBDEs in Sediment and Fish Samples from Brackish and Coastal Waters off Savannah, Georgia, USA** D. Benningfield, B.M. Cassidy, Murray State University / Chemistry and Watershed Studies Institute; J.P. Richardson, Savannah State University / Department of Natural Sciences and Mathematics; K.S. Sajwan, Savannah State University; B.G. Loganathan, Murray State University / Department of Chemistry & Watershed Studies Institute, Murray State University / Department of Chemistry and Watershed Studies Institute. Persistent organohalogen pollutants are of great concern due their negative effects on environment, wildlife and human health. Estuaries and coastal marine environments are considered the most sensitive areas to the accumulation of these pollutants. Objective of this study was to understand the contamination status of classical and emerging pollutants in the coastal waters off Savannah, Georgia. PCB congeners, chlorinated pesticides and PBDEs were analyzed in sediment and various fish species samples collected from riverine (Vernon River, Harmon Canal) and coastal waters off Savannah, Georgia. Standard analytical procedures were followed to determine the concentrations of these contaminants. Results revealed that detectable levels of PCBs, pesticides and PBDEs were found in sediments and fish samples from this region. The results were compared with the earlier studies on these pollutants to discern temporal trends.

**TP275 Persistent organic pollutants in indoor air: assessing extraction method** O. Junior, C. Lourencetti, M. Marchi, Unesp. Regarding the properties of the compounds classified as Persistent Organic Pollutants

(POPs), their harmful impact to humans is a concern for the Stockholm Convention parties. Breast milk and ambient air have been recommended to be analyzed in order to determine the POP levels and the human exposition risk to these compounds. Although efforts to control POPs production and release to the environment have been made, data about their levels in Brazil are still scarce, mainly in the atmosphere or indoor environment. Considering the permanence of people inside its house, this study presents a method for POPs extraction from sorbent employed in indoor air sampling, with identification and quantification by GC-ECD. Extraction by shaking of PCBs congeners (28, 52, 101, 118, 138, 153 and 180), DDTs and endosulfans, including their metabolites (DDD, DDEs and endosulfan sulfate) from XAD-2 cartridges (270/140 mg sorbent) were compared employing hexane (2 mL) and hexane:acetone (1:1) (2 mL). Hexane showed to be the best selective and efficient extractor solvent with accuracy ranging from 56 to 121% and from 84 to 153 % for both spiked level. Precision was smaller than 30% for all cases. Not satisfactory result was obtained for PCB 28 and PCB 52 for the smallest spiked level. Employing sampling flow of 2 L/min during 12 hours, the presented method is able to determine approximately 10.4 ng/m<sup>3</sup> to 20.8 ng/m<sup>3</sup> of individual studied POPs in indoor air. This method will be applied to assess human exposition in a tropical area of the Southeast of Brazil with a historic use of DDT and a still intensive use of endosulfan.

**TP276 Persistent Organic Pollutants in the Plasma of Loggerhead Sea Turtles in Florida** J. Hogue, National Institute of Standards and Technology (NIST) / Analytical Chemistry Division; B. Schroeder, Office of Protected Resources, National Marine Fisheries Service; D. Bagley, University of Central Florida; A. Foley, Fish and Wildlife Research Institute; J.M. Keller, National Institute of Standards and Technology. Persistent organic pollutants (POPs) are globally distributed contaminants detectable in blood of several wildlife species. The loggerhead sea turtle (*Caretta caretta*), a long-lived species, is listed as threatened under the Endangered Species Act. Previous studies have shown *C. caretta* to be a good bioindicator species for regional-scale coastal contamination. This study examined a suite of polychlorinated biphenyls (PCBs), organochlorine (OC) pesticides, polybrominated diphenyl ethers, and hexabromocyclododecanes, in plasma of 37 immature loggerhead sea turtles from two locations not previously tested for POPs and where fibropapillomatosis (FP) has been documented in sea turtles: Indian River Lagoon (IRL) ( $n=15$ ) and Florida Bay (FL Bay) ( $n=22$ ), Florida, USA. Samples were extracted using open focused microwave, and compounds were analyzed using gas chromatography/mass spectrometry. A preliminary analysis was conducted on PCB-153 and 4,4'-DDE, two of the most predominant POPs shown in previous sea turtle studies. For PCB-153, means (and standard error) in pg/g wet mass were 411 (80) for IRL and 277 (76) for FL Bay; and for 4,4'-DDE, they were 125 (26) for IRL and 21.3 (8.2) for FL Bay. IRL turtles had significantly higher concentrations of 4,4'-DDE than FL Bay turtles ( $p < 0.001$ ). The presence of external FP tumors was documented in 20% of IRL and 18% of FL Bay turtles that were sampled for this study. However, PCB-153 and 4,4'-DDE concentrations were not significantly higher in the tumored turtles (respective means in pg/g: 312 and 65.9 for IRL and 318 and 49.1 for FL Bay). This finding suggests that these two contaminants alone do not seem to influence the expression of the disease, but power was low ( $\approx 9$ -38%) to detect differences. This study provides the first baseline concentrations of PCBs and OC pesticides in blood of sea turtles from inshore waters of Florida, and is the first to investigate these compounds in relation to FP in loggerhead turtles.

**TP277 Polybrominated diphenyl ethers (PBDEs) and hydroxylated metabolites in paired maternal and cord serum samples** A. Chen, University of Cincinnati; J. Park, California Environmental Protection Agency / Department of Toxic Substances Control, California Environmental Protection Agency / Department of Toxic Substances Control, Environmental Chemistry Laboratory, California Environmental Protection Agency / Research Scientist; L. Linderholm, California Department of Toxic Substances Control; A. Rhee, California Department of Toxic Substances Control; M. Petreas, California Department of Toxic Substances Control, DTSC; E. DeFranco, K. Dietrich, S. Ho, University of Cincinnati. Polybrominated diphenyl ethers (PBDEs) were widely used as flame retardants in the past three decades. These compounds are lipophilic and easily cross the placenta from mother to fetus. It is not clear whether hydroxylated PBDEs (OH-PBDEs), metabolites of PBDEs with more structural similarity to thyroid hormones but more hydrophilic, differ in maternal and cord blood samples.

**Methods:** We analyzed both PBDEs (BDE-28, -47, -99, -100, -153, -154, -209) and OH-PBDEs (6-OH-BDE-47, 5-OH-BDE-47, 4'-OH-BDE-49, 5'-OH-BDE-99) in a pilot of 20 pairs of maternal and cord serum samples collected in Cincinnati, OH in 2011. We used standard liquid-liquid serum extraction and phase separation techniques. Analysis of PBDE was performed by gas chromatography/high resolution mass spectrometry (GC-HRMS, Thermo-Finnigan) coupled with isotope dilution. OH-PBDEs were determined as methyl derivatives (MeO-PBDEs) by using Varian 1200 GC/MS with negative chemical ionization mode. **Results:** The geometric mean concentration of  $\Sigma$ OH-BDEs (the sum of four OH-PBDEs) was 49.76 pg/ml in cord sera, higher than 32.84 pg/ml in maternal sera. Similarly, cord serum total BDEs had a higher geometric mean than maternal serum samples (45.51 vs. 32.07 ng/g lipid). The ratio of cord to maternal (C:M) total BDEs had a mean of 1.59 (SD 0.94, range 0.91 to 4.12), while the mean C:M ratio of total OH-BDEs was 1.69 (SD 0.83, range 0.57 to 3.55). The maternal and cord total OH-BDEs were inversely associated with birth weight while total BDEs did not reach statistical significance. **Conclusion:** The study suggests fetuses may receive higher PBDE and OH-PBDE exposure compared with their mothers and these prenatal exposures lower birth weight. The role of OH-PBDEs on fetal hormonal profile and neurological development warrants further studies.

**TP278 Synthesis and Evaluation of Naturally Occurring Halogenated Bipyrroles in a Marine Relevant Species, *Fundulus heteroclitus*** K.S. Foster, University of Mississippi / Environmental Toxicology Research Program, University of Mississippi / Department of Medicinal Chemistry; K.L. Willett, University of Mississippi / Environmental Toxicology Research Program; J.M. Rimoldi, University of Mississippi / Professor of Medicinal Chemistry, University of Mississippi / Department of Medicinal Chemistry. The occurrence halogenated dimethyl-2,2'-bipyrroles (HDBP's) by nature have recently been discovered in seabird eggs bioaccumulating in trophic organisms. These compounds are suspected to be biogenic in nature from evidence of marine natural products that are persistent in the environment and widespread in the Pacific and Atlantic Ocean. These compounds exhibit chlorinated and brominated substitution patterns that closely resemble anthropogenic pollutants like the polychlorinated biphenyls (PCBs), polybrominated biphenyls (PBBs), and polybrominated diphenyl ethers (PBDEs). Environmental health concerns continue to surface regarding these naturally occurring HDBP's since several of these analogs were shown to induce the cytochrome P4501A (CYP1A) gene through activation of the AhR signaling pathway, similar to other anthropogenic pollutants, and also resulted in porphyrin accumulation in chicken embryo hepatocytes. Based on these studies, we have focused our efforts on synthesizing and examining the cellular and subcellular toxicity of three HDBP's (DBP-Br<sub>6</sub>, DBP-Cl<sub>6</sub>, DBP-Br<sub>4</sub>Cl<sub>2</sub>) in a marine relevant species, *Fundulus heteroclitus* for the induction of the cytochrome P4501A (CYP1A) gene through activation of AhR signaling pathway. *Fundulus* are considered to be the "premier teleost model" in marine biology research, having several functionalized genes characterized specifically to this fish model capable of tolerating exposure to many environmental contaminants. Exposure of three HDBP's to *Fundulus* revealed that several of these analogs exhibited significant AhR-mediated effects, when compared to the positive control PCB-126.

**TP279 The Influence of High Molecular Weight Polymers in Biosolids on the Presence and Fate of Nutrients, Heavy Metals, and Anthropogenic Organic Compounds** M.K. Dunbar, J.S. Carsella, Colorado State University-Pueblo / Chemistry; C.A. Kinney, Eastern Washington University / Chemistry & Biochemistry, Colorado State University-Pueblo / Chemistry. Treatment of municipal wastewater results in the production of sewage sludge. If the sludge meets regulatory requirements, it can be classified as biosolids, a nutrient rich solid that can be applied to agricultural or reclamation land as fertilizer. High molecular weight cationic polymers (HMWPs) are commonly used to promote flocculation during sludge dewatering. Five commonly used HMWPs, four acrylamide/allyl triethyl ammonium chloride co-polymers (20, 40, 60, and 80 mole % cationic) and an aminomethylated polyacrylamide co-polymer, were tested to determine their effect on nutrient (phosphate, nitrate, and ammonium) availability, leaching of heavy metals, and the presence and fate of anthropogenic organic contaminants (AOCs). The cationic character of the HMWP limited the leachability of phosphate in biosolids samples. The control biosolids (no HMWP) had a higher quantity of leachable phosphate (organic and inorganic), 10.58 mg/g, compared to the biosolids containing HMWPs, 0.79-3.19 mg/g. This is due

to a decrease in the quantity of leachable organic and inorganic phosphate in the biosolids containing HMWP; yet all of the biosolids samples contained about 2% phosphorous on a mass basis. Leachable ammonium increased with the cationic strength of the acrylamide/allyl triethyl ammonium chloride co-polymers used to treat four of the biosolids samples, ranging from 1.14 to 1.97 mg/g; while 0.48 mg/g leached from the biosolids treated with the aminomethylated polyacrylamide co-polymer and 0.19 mg/g leached from the control biosolids. All of the biosolids samples contained about the same amount of total nitrogen, an average of 47.45 mg/g. Studies into the effects of the HMWPs on the quantity of leachable nitrate and heavy metals as well as the presence and availability of AOCs in biosolids are ongoing and the results will be forthcoming.

**TP280 US EPA Decabromodiphenyl ether alternatives assessment results** M. Kawa, J. Rhoades, SRC Inc.; E.T. Lavoie, US Environmental Protection Agency / Design for the Environment Program, Environmental Protection Agency / Office of Pollution Prevention and Toxics, U.S. Environmental Protection Agency / Toxicologist; C. Baier-Anderson, US Environmental Protection Agency / Design for the Environment; J. Tunkel, SRC, Inc. / Chemical, Biologica, Environmental Center. The U.S. Environmental Protection Agency (US EPA) Design for Environment (DfE) Program undertook a chemical alternatives assessment for decabromodiphenyl ether (decaBDE) as part of the Action Plan for Polybrominated Diphenyl Ethers (PBDEs) published in December 2009. The alternatives assessment was done via a multi-stakeholder partnership that identified 30 functional alternatives to decaBDE for a variety of uses. The hazard assessment for decaBDE and the alternatives used the DfE hazard evaluation criteria to assign hazard designations for human health toxicity, ecological toxicity and environmental fate endpoints. The alternatives included a range of flame retardant chemistries including halogen-free organic substances, inorganic materials, polymeric and non-polymeric substances and novel halogenated substances. Some alternatives were well characterized for all endpoints. Other alternatives were poorly characterized, wherein analog data, predictive models, structural alerts and expert judgment were used to make hazard designations for data gaps. Trends for human health, ecological toxicity and fate characteristics were indicated in a number of the alternatives. In general, molecular size ranges, molecular structures, and/or functional groups were found to be most influential on the hazard designations. A novel component of this assessment was the evaluation of higher molecular weight polymers for their human health, ecological toxicity, persistence, and bioaccumulation hazards. Effective hazard assessment approaches, coupled with decision-making protocols that are practical tools for businesses to use in materials selections, will contribute to more sustainable product development when human health or ecological toxicity concerns exist. The resulting hazard profiles should be of value to manufacturers making substitution decisions in preparation for the upcoming decaBDE phase out.

**WP001 Plastic ingestion and chemical contamination in yellowtail (*Seriola lalandi*) from the North Pacific Central Gyre** M. Gassel, California Environmental Protection Agency / Office of Environmental Health Hazard Assessment; S. Harwani, California Environmental Protection Agency / Department of Toxic Substances Control, California Environmental Protection Agency / Department of Toxic Substances Control, Environmental Chemistry Laboratory, California Environmental Protection Agency / Department of Toxic Substances Control; J. Park, California Environmental Protection Agency / Department of Toxic Substances Control, California Environmental Protection Agency / Department of Toxic Substances Control, Environmental Chemistry Laboratory, California Environmental Protection Agency / Research Scientist; A. Jahn, Kier Associates. Plastics have become the mainstay of commonplace products including everything from automobiles to chip clips, building materials, clothing, household appliances, and electronic equipment, to name a few. A number of chemicals, including PCBs, PBDEs, and nonylphenol, have been added to plastics as plasticizers, stabilizers, and flame retardants to manage the structure and behavior of the polymers and the inherent flammability of petroleum-derived polymers. Inadequate life cycle management of plastic products is contributing to the increasing accumulation of plastics in the environment and ocean gyres, in particular. Furthermore, evidence has shown that hydrophobic contaminants adsorb to plastic debris in the ocean. Concerns for transfer of plastic-associated contaminants to the food web have been raised, but it has been challenging to differentiate contributions to food web contamination from exposure via consumption of prey versus plastic. In this study, we analyzed stomach contents and measured PCBs, DDTs and other organochlorine pesticides, PBDEs, and the nonionic surfactants, specifically, nonylphenol ethoxylates, in young yellowtail collected from the North Pacific Central Gyre. Ingestion of synthetic debris was confirmed in approximately 10% of the population. Mean total PCBs and DDTs were 352+240 ng/g lipid weight (lw) and 1425+1118 ng/g lw, respectively. Mean total PBDEs was 8.21+10.9 ng/g lw, with BDEs 47, 209, and 99 representing 89% of total analyzed PBDEs. We compared chemical concentrations and congener profiles in yellowtail tissues to concentrations in fish from various geographic regions and to results from analysis of plastic debris from the gyre. Concentrations of PCBs, OCPs, and PBDEs were higher than might be expected in pelagic fish from such a remote location and were similar to those reported for some fish from certain populated coastal areas and deep-sea fish. We also found similarities to key congeners in plastic debris suggestive of plastic as a possible source of exposure. Moreover, the major by-product of the surfactants, nonylphenol (NP), was detected in about one third of the yellowtail (167+72.1 ng/g wet weight, excluding non-detects). Because NP in the environment is strongly associated with sewage treatment effluents and industrial discharge, is unlikely to undergo long-range transport to the gyre, and has been measured in plastic samples from the gyre, we concluded that plastic-mediated exposure best explained our findings of NP in yellowtail.

**WP002 Factors affecting leaching rates of phthalates and bisphenol A from canine training devices** K.J. Wooten, Texas Tech University / TIEHH; P.N. Smith, Texas Tech University / Environmental Toxicology, Texas Tech University / Institute of Environ. & Human Health. Pet canines exhibit chewing and mouthing behaviors that may lead to oral exposures to chemicals present in their everyday environment. The objective of this study was to examine the potential for plastic bumpers, canine training devices, to leach phthalates and bisphenol A (BPA). Target phthalates included dimethyl (DMP), diethyl (DEP), dibutyl (DBP), benzylbutyl (BBP) and di-2-ethylhexyl (DEHP) phthalate. Canine exposure scenarios were simulated in the laboratory by immersion of bumpers into synthetic canine saliva. Bumper brand, color, presence/absence of chewing, and presence/absence of aging via outdoor storage were evaluated for effects on the quantity of phthalates and BPA leaching into saliva. Quantification of phthalates and BPA was done by LC-MS/MS. Total concentration of plastic additives was significantly affected by both color ( $p=0.039$ ) and treatment (new vs. aged vs. chewed,  $p=0.005$ ). When concentrations of individual plastic additives were considered, significant ( $p<0.05$ ) effects of brand (DEP, DEHP, BPA), color (DEP, DBP, BPA), and treatment (DEP, BBP, BPA) were observed. Overall, this study suggests that products marketed for canine use can lead to the exposure of pets to endocrine disrupting chemicals, and that routine storage and usage can affect concentrations of these chemicals that leach into canine saliva.

**WP003 Measuring bioaccumulation of POPs sorbed to plastic marine debris using a medaka fish model** R. Struch, San Diego State University / Graduate School of Public Health; C. Rochman, San Diego State University / Department of Ecology; E. Hoh, San Diego State University / Graduate School of Public Health; S. Teh, University of California Davis / Aquatic Health Program. Plastic marine debris has become a contaminant of concern in many marine ecosystems due to its ubiquity and ability to serve as a medium for concentrating persistent organic pollutants (POPs). Several marine organisms have been shown to directly ingest plastic debris, potentially providing another pathway for bioaccumulation. The purpose of this study was to measure contaminant transfer from plastic to fish through dietary exposure using plastic marine debris and golden medaka (*Oryzias latipes*). Adult medaka were divided equally into nine tanks randomly assigned to one of three dietary treatments ( $n=3$ ): control, virgin plastic, and ocean plastic. Virgin and ocean plastic treatments were prepared by substituting 10% of the control's binder (casein) with "virgin" low-density polyethylene (LDPE) or "ocean" LDPE, which had been deployed in San Diego Bay for four months. Analyses of the diets indicated PAHs were the most prevalent and concentrated contaminants in virgin and ocean plastic, but PCBs, pesticides, and PBDEs were also present in ocean samples. At one and two month periods, ~5 g medaka wet weight were sampled for body burden of PAHs and their metabolites, PCBs, organochlorine pesticides, and PBDEs. Results to date will be presented at this meeting, but we hypothesize medaka fed ocean plastic will have greater concentrations of POPs than medaka fed virgin plastic or control diets. This study aims to provide baseline data for estimating contaminant transfer from chronic exposure to plastic marine debris in fish. Understanding the role of plastics as a vessel for bioaccumulation of POPs may better inform waste policy and mitigate oceans and human health risks.

**WP004 International Pellet Watch : Global distribution of POPs, their temporal trends, and ecotoxicological significance** H. Takada, M. Yuyama, R. Yamashita, M. Ito, Y.B. Geok, J. Hosoda, Tokyo University of Agriculture & Technology / Laboratory of Organic Geochemistry (LOG). International Pellet Watch (IPW) is global monitoring of persistent organic pollutants (POPs) by using plastic resin pellets. IPW started in 2005 and pellets from ~ 120 locations from 40 countries have been analyzed for PCBs, DDTs, and HCHs. Hot spots of PCB pollution were identified, e.g., Le Havre (France), Athens (Greece), Tokyo Bay (Japan), Sydney Harbor (Australia), Los Angeles, San Francisco, Boston Harbor, New York Bight, Lake Erie (USA), Santos Bay (Brazil). Combination of IPW with passive air samples (PAS) indicates that legacy pollution is major contributor to most of the hot spots of PCB pollution. Higher concentrations of HCHs were observed at South Durban (South Africa), Maputo (Mozambique), and Queenscliff (Australia). At South Durban and Maputo, higher concentrations of HCHs were observed in air through the analysis of PAS, indicating current usage of HCH pesticide in the area. In Tokyo Bay and California coast, time-series sampling was conducted. At several locations on California coasts, decreasing trend in PCB concentrations was observed from 2006 to 2011. On the other hand, no decrease was observed in Tokyo Bay from 2003 to 2011. The difference may be related to geographical difference between the two coastal zones (semi-enclosed system vs. open system). IPW tells us the risks associated with chemicals in marine plastics, too. In IPW we always analyze 5 pools from each location and have found some pools with sporadically high concentrations of POPs even in some remote areas. In IPW we take median concentration to exclude sporadic high concentrations and to get a representative pollution status for individual locations. However, marine organisms cannot take median doses but they take everything. Thus, the plastic sample with sporadically high concentration of POPs poses a serious threat to marine organisms. In remote areas wild animals are exposed to minimal amounts of POPs through natural media (water, air, and food web). These wild lives are also vulnerable to the threat of POPs through sporadic high concentrations. The existence of sporadic high concentrations of POPs is a unique but hazardous aspect of marine plastics.

**WP005 Occurrence and distribution of polycyclic aromatic hydrocarbons (PAHs) in plastic pellets sampled in depth in a sandy beach** M. Fisner, S. Taniguchi, M.C. Bicego, A. Turra, University of São Paulo. Plastic pellets have the ability to adsorb organic pollutants, such as polycyclic aromatic hydrocarbons (PAHs). Due to characteristics such as natural resistance to degradation and buoyancy these polymers are transported in the marine environment, and they are usually found buried on the sandy beaches. In



this way, the present study analyzed the occurrence and distribution of PAH adsorbed in plastic pellets sampled in a cubic meter of sediment. Sampling was performed in a sandy beach of Santos Bay, Brazil, located near two potentially polluting areas – the industrial complex (Cubatão) and the major port of Latin America (Porto de Santos). Samples of pellets were taken every 10 cm of sand, up to 1 meter deep. After that, samples were Soxhlet-extracted, and the extract was cleaned up by adsorption chromatography using a column of silica and alumina both 5% deactivated. PAH compounds were identified and quantified by a gas chromatograph with mass spectrometer (GC/MS). The PAH concentration varied between 385 and 1,996 ng g<sup>-1</sup>, with the highest concentration in the surficial layer followed by layers of 60-70 cm and 30-40 cm, respectively. Given that plastic pellets could be lost from many places (contaminated or not) and, there is evidence that aged pellets (which stayed longer in the environment) show highest concentration of pollutants; these factors should be considered in future sample designs. As well as the observed variability, allowing the comparison between different areas. The compounds which showed major concentrations were those with high molecular weight and alkylated ones, such as benzo(g,h,i)perylene, indeno[1,2,3-c,d]pyrene, benzo(e)pyrene and S-dimethylchrysenes. These compounds indicate the input of petrogenic and pyrolytic sources, in the same way that occurred in plastic pellets collected in the surface of sandy beaches of Santos Bay. It suggests that there is no difference between the sources of contamination of PAH in plastic pellets from surface or depth.

**WP006 Exploring Opportunities and Obstacles in Society's Transition to Environmentally Sustainable Plastics** E.J. North, Arizona State University / Center for Environmental Security; R.U. Halden, Arizona State University / Center for Environmental Security, The Biodesign Institute at Arizona State University. Due to the persistence of plastic debris in the environment as well as potential health risks from compounds used in plastic manufacture, efforts have been made to engineer biodegradable alternatives. This paper describes the different types of biodegradable plastics that have been developed and explores barriers to their use as sustainable alternatives to consumer products made from moldable petroleum-based polymers. Important factors to be considered when evaluating biodegradable plastics are their cost relative to conventional plastics and the conditions necessary for timely biodegradation to avoid their accumulation in the environment. In addition, consumer awareness of the meaning of product labels such as 'biodegradable' and 'recyclable' are discussed as only one of multiple human factors determining the extent and speed at which our society will transition from existing consumption patterns to using more sustainable biopolymers of more balanced environmental impact.

**WP007 Hg bioaccumulation in shoots of the macrophyte *Elodea nuttallii*: an opportunity for phytoremediation or a risk for the trophic chain?** N. Regier, C. Cosio, University of Geneva. Previous studies suggest that macrophytes might participate in bioaccumulation and biomagnification of toxic mercury (Hg) in aquatic environment. Hg bioaccumulation and uptake mechanisms in macrophytes need therefore to be studied. Amongst several macrophytes collected in an Hg contaminated reservoir in Romania, *Elodea nuttallii* showed a high organic and inorganic Hg accumulation and was then further studied in the laboratory. Tolerance and accumulation of Hg this plant was also high in the microcosm. Basipetal transport of inorganic Hg was predominant, whereas acropetal transport of methyl-Hg was observed with apparently negligible methylation or demethylation *in planta*. Hg concentrations were higher in roots>leaves>stems and in top>middle>bottom of shoots. In shoots, more than 60% Hg was found intracellularly where it is believed to be highly available to predators. Accumulation of inorganic and methyl-Hg in shoots was highly reduced by cold, death and by competition with Cu<sup>+</sup> for inorganic Hg. Hg in *E. nuttallii* shoots seems to mainly originate from the water column, but methyl-Hg can also be remobilized from the sediments and might drive in part its entry in the food web. At the cellular level, uptake of Hg into the cell sap of shoots was linked to the metabolism, in particular to copper transporters. The present work highlights an important breakthrough in our understanding of Hg accumulation and biomagnifications: the remobilization of methyl-Hg from sediments to aquatic plants and differences in uptake mechanisms of inorganic and methyl-Hg in a macrophyte. Our results revealed that shoots of *E. nuttallii* are extremely tolerant to Hg and show huge bioaccumulation ability. Both might be interesting for phytoremediation of contaminated water. Based on densities previously reported in the field (350-2800 g DW m<sup>-3</sup>) and concentration of Hg found in Babeni reservoir, it can be estimated

that 0.7 to 5.6 mg Hg m<sup>-3</sup> could be removed with these plants. On the other hand, the Hg uptake by shoots might be an important pathway of Hg from the water to organisms feeding on the macrophytes. In conclusion, the presence of these plants should not be overlooked in contaminated sites.

**WP008 Toxicity and uptake of mercury by unicellular green algae in presence of synthetic organic compounds** S. Le Faucheur, University of Geneva / Institute F.-A. Forel; C. Portilla Castillo, V. Slaveykova, University of Geneva. Phytoplankton plays a critical role in the biogeochemical cycle of mercury (Hg) due to its position at the base of the trophic chain and its capacity to biologically transform Hg. Consequently, it is crucial to understand which parameters control phytoplankton's Hg uptake in order to establish adequate water quality criteria. In natural waters, algae are exposed to mixtures of inorganic and organic contaminants, which can be potentially more toxic than the exposure to one contaminant at a time. Here, we are interested in studying the effects of perfluorooctane sulphonate (PFOS), a global pollutant with surface-active properties, on Hg uptake by unicellular green algae. Our working hypothesis is that simultaneous exposure of phytoplankton to Hg and PFOS will enhance the uptake of Hg and thus its toxicity for the algae. To that aim, *Chlamydomonas reinhardtii* was exposed to increasing concentrations of HgCl<sub>2</sub><sup>0</sup> and PFOS alone and in combination. Growth of *Chlamydomonas reinhardtii* and the modes of action of the studied contaminants were examined using flow cytometry. Modification of membrane permeability was assessed using propidium iodide, a fluorescent dye that only permeates through damaged membranes, whereas production of reactive oxygen species was evaluated using CM-H<sub>2</sub>DCFDA, a general oxidative indicator. Uptake of HgCl<sub>2</sub><sup>0</sup> by *C. reinhardtii* in the presence and absence of PFOS were analyzed after short-time exposure (t < 1 hour) to the studied contaminants. Intracellular Hg content was measured by an Advanced Mercury Analyser (AMA 254) after washing algal cells with cysteine. At each treatment, Hg uptake rates were calculated and compared with each other to determine the effect of PFOS. Algal growth remains optimal up to concentrations as high as 10<sup>-7</sup> M HgCl<sub>2</sub><sup>0</sup> and 10<sup>-5</sup> M PFOS. However, exposure to a mixture of 10<sup>-7</sup> M HgCl<sub>2</sub><sup>0</sup> and 10<sup>-7</sup> M PFOS leads to 20 % inhibition of algal growth, which is unexpected given the inhibition rates obtained in exposure experiments with only one toxicant. Preliminary results show that 10<sup>-7</sup> M PFOS does not induce any changes in the membrane permeability of *C. reinhardtii*. The results obtained in this study are expected to improve our understanding of mercury-phytoplankton interactions in order to better protect aquatic wildlife.

**WP009 Improving Mercury-Based Consumption Advisories for Lake Trout (*Salvelinus namaycush*) from Large Lakes of the Western US** W. Smiley, T. Saddler, Salish Kootenai College / Department of Life Sciences; D.K. Stevens, Salish Kootenai College / Department of Life Sciences, Salish Kootenai College / Dept Life Sciences. Mercury is a potent environmental toxin known to cause neurodevelopmental deficits in children, and cardiovascular effects in adults. The major route of exposure to mercury is as methylmercury (MeHg) through consumption of fish. Epidemiological studies of fish-eating populations indicate there may be dietary factors capable of offsetting risk from MeHg exposure, such as the n-3 fatty acids and selenium content of the fish. Lake trout (*Salvelinus namaycush*) are long-lived, highly predatory fish and have been shown to biomagnify Hg to high concentrations in a length-dependent manner. Lake trout have been introduced in many large lakes in the western US as a sport fish and are now threatening native fish species. One management tool to decrease lake trout populations is to promote large-scale catch and consumption that includes donation to area food banks. Consumption advisories generally include number of meals per month for various length brackets for two populations of consumers, sensitive populations, (children, pregnant and/or nursing women), and the general public. These guidelines do not consider possible modulating factors, such as n-3 fats or Se. Advisories for lake trout from Lake Pend Oreille, Idaho (LPO) lack a length-based system, and offer advice based solely on the number of fish consumed per month. The advisory for lake trout from Yellowstone Lake, Wyoming (YSL) uses a length-based guideline, but lacks separate advisories for each population. Both of these advisories are based on a small sample of fish. To determine if these current advisories for LPO and YSL afford adequate protection, total Hg, n-3 fatty acids and Se concentrations were determined in muscle samples of 50 lake trout of varying lengths from each lake. Hg levels in the lake trout from YSL were significantly lower than those from LPO (p < 0.05). In lake trout from both lakes, Se concentration was found to be independent of length

and n-3 fats increased with length, but at a lower rate than the Hg increase. Based on recently proposed models that have attempted to include the effects of n-3 fatty acids and Se into the mercury risk assessment process, neither of these two factors, therefore, would appear offer realistic protection from the Hg found in the larger fish. By integrating these factors, more complete length-based consumption advisories for lake trout from LPO and YSL have been proposed.

**WP1010 Synthesis of Global Biotic Mercury Concentrations to Examine Spatial and Temporal Patterns from Existing Data** M. Turnquist, State University of New York – College of Environmental Science and Forestry / Environmental Forest Biology, Biodiversity Research Institute; D.C. Evers, A. Zyer, Biodiversity Research Institute. Mercury (Hg) is an increasingly problematic global pollutant as its use in artisanal small-scale gold mining and release through coal-fired power plants is growing. To best manage and control Hg it is important to understand its spatial distribution and magnitude in foods important for human consumption, including fish and marine mammals. The identification of biological Hg hotspots will have policy ramifications, including the legally binding agreement for Hg being developed by the United Nations Environment Programme. We examined 192 peer-reviewed papers with samples collected from 1969-2010. Samples were divided into nine major oceanic regions, as well as fresh water samples. The aggregation of data focused on the mean Hg concentration by major taxa type at each unique location. Teleosts fish ( $n = 12,384$ ), elasmobranchs ( $n = 6,492$ ), and marine mammals ( $n = 4,108$ ) were the taxonomic focal groups examined [ $n =$  the total number of individuals sampled] at 426 unique locations. Geographic comparisons are possible with these robust sample sizes, although we have not controlled for differences in species and age classes, therefore only broad spatial patterns can be distinguished. Marine teleost or boney fish exhibited significant differences in average muscle Hg concentrations among nine oceanic regions ( $p < 0.001$ ), with the highest average being in the Gulf of Mexico-Caribbean (1.24 ppm, ww), and the lowest Hg concentration in the Antarctic (0.02 ppm, ww). Through time, there were significant increases in Hg concentrations in the North Pacific and Indian oceanic regions ( $p < 0.05$ ). There were significant differences in average muscle Hg concentrations for elasmobranchs among nine oceanic regions ( $p = 0.004$ ), with the highest being in the Mediterranean (3.00 ppm, ww), and the lowest average Hg concentration in the South Pacific (0.96 ppm, ww). Overall, there was a slightly significant increase in elasmobranch muscle Hg concentrations over the time period of 1977 to 2008 ( $p$ -value = 0.068), but no significant time trends by region. For toothed whales there were significant differences in average muscle Hg concentrations among the nine oceanic regions ( $p < 0.001$ ), with the highest in the Mediterranean (13.15 ppm, ww) and the lowest average Hg concentration in the Gulf of Mexico-Caribbean (0.93 ppm, ww). Only the Arctic region had a significant change in Hg through time, where there was an increase from 1981 to 2008 ( $p = 0.005$ ).

**WP1011 Methylmercury Exposure in Women of Child-bearing Age on the Flathead Indian Reservation, MT, USA** T. Saddler, W. Smiley, Salish Kootenai College / Department of Life Sciences; S. Kuntz, Montana State University / College of Nursing; D.K. Stevens, Salish Kootenai College / Department of Life Sciences, Salish Kootenai College / Dept Life Sciences. The main route of exposure to mercury is as methylmercury through consumption of fish. Methylmercury has been linked to neurodevelopmental deficits in young children and children exposed in-utero, as well as cardiovascular disease in adults. Pregnant women, women who may become pregnant, women who are nursing and young children represent the most sensitive population to the negative neurodevelopmental effects of mercury. Thousands of lake trout are donated to Flathead Reservation food banks through a local fishing tournament designed to reduce lake trout populations through a large scale catch and consumption strategy (called "Mack Days"). Previous studies of lake trout from Flathead Lake, Montana, have shown elevated levels of methylmercury, resulting in updated fish consumption guidelines. Participants in the Women, Infant and Children program (WIC) are eligible to receive free fish from local food banks, which may include these lake trout. A previous survey of local women in the WIC program showed that approximately 50% eat fish, however, the majority were not aware of potential adverse effects of mercury or of any fish consumption advisories. Since dietary surveys are frequently inaccurate, follow-up hair mercury analysis was necessary to determine if any of these participants were at risk from elevated methylmercury exposure. The survey was also used to

better develop mercury risk communication strategies in this population. In this study, hair samples were collected from 183 women of child-bearing age (18-40) from around the Flathead Indian Reservation, most of whom were WIC participants. Mercury levels were determined in samples of hair 3 cm in length (20-25 mg) from the scalp, representing a 3-month integrated exposure, using EPA method 7473. Results demonstrate that the hair levels in ~90% of the participants sampled were below the national average of 200-300 ppb mercury, some even below 50 ppb, indicating one or fewer fish portions per month. None were above the 1000 ppb threshold of concern. Longitudinal analysis of a subset of samples shows this consumption pattern to be fairly consistent over the course of past the year. This shows that, although some women on the reservation are offered free fish, many do not take advantage of this resource. The data suggests that lack of dietary omega-3 oils from not eating enough fish may pose a greater risk than the deleterious effects of mercury from eating too much fish.

**WP1012 Application and validation of the National Descriptive Model of Mercury in Fish (NDMMF)** M. Brigham, M. Fienen, D. Donato, U.S. Geological Survey; S. Wente, U.S. Environmental Protection; D. Lorenz, U.S. Geological Survey. The National Descriptive Model of Mercury in Fish (NDMMF; Wente, S. P., 2004, U.S. Geological Survey Scientific Investigation Report 2004-5199, <http://pubs.usgs.gov/sir/2004/5199/>) is a powerful tool for analyzing fish-mercury data sets with many observations. The NDMMF accounts for variations in fish-mercury concentrations due to species, sampled cut (for example, skin-off fillet, whole body, etc.), fish length, and event (defined as all fish samples collected at a site during a given year). The NDMMF facilitates analysis of the distribution of mercury in freshwater fish by allowing standardized comparisons among sampling events (that is, among sites and over time). We applied the NDMMF to a data set of 101,839 observations of fish-mercury concentrations compiled from numerous state, federal, and tribal monitoring programs. The data set spans the years 1967-2005, and includes data from more than 10,000 sites across 49 states in the United States. The median standard error of prediction was 10.3 percent (expressed as a percentage of concentration). The median ratio of model-predicted to measured fish-mercury concentration was 0.94, indicating low bias; the 10th and 90th percentiles of this ratio were 0.48 and 2.0, respectively, meaning that the central 80 percent of predicted values ranged from about half to twice the measured value. A leave-one-out cross-validation study was conducted to gain a better understanding of model performance in relation to different characteristics that could affect model predictions. Low bias (median residuals close to zero) was observed for most fish species (all cuts combined), and most unique species-cut combinations. In addition, low bias was observed for nearly every State represented in the data base, the exception being one state that used a high detection limit for a number of years.

**WP1013 How does temperature affect herbicide toxicity in phytoplankton?** A. Chalifour, University of Quebec in Montreal; M.T. Arts, Environment Canada / National Water Research Institute; M.J. Kainz, Inter-university Research Center WasserCluster Lunz – Biological Station / Aquatic Lipid and Ecotoxicology Research Group (LIPTOX); P. Juneau, University of Quebec in Montreal / Department of Biological Sciences-TOXEN. Temperature is known to affect cell division, photosynthesis, enzymatic activity, pigment content and lipid composition of aquatic microorganisms. Temperature may also interact with herbicides to modify toxicity effects. Sensitivity of algae and cyanobacteria to herbicides is related to the specific temperature optima of each species and the ability of cells to acclimate to various temperatures. In a first set of experiments, *Scenedesmus obliquus*, *Navicula pelliculosa* and two strains of *Microcystis aeruginosa* were acclimated to 10, 15 and 25°C and exposed to the photosynthesis-inhibiting herbicide atrazine. *S. obliquus* and *M. aeruginosa* were more sensitive to atrazine at low temperature, while temperature did not change the sensitivity of *N. pelliculosa* to atrazine. The ability of the microorganisms to cope with a high excitation pressure, by increasing its protective carotenoid content and non-photochemical energy dissipation is associated with the observed differences in sensitivity. In a second study, *Chlamydomonas reinhardtii* was acclimated to 8, 15 and 25°C and was exposed to two bleaching herbicides, norflurazon and fluridon. While *C. reinhardtii* was more sensitive to both herbicides at 15°C than at 25°C, cells were very tolerant to norflurazon and fluridon at 8°C. Changes in antioxidant enzymatic activity, photoprotective pigment and polyunsaturated fatty acid content are all involved in this increased tolerance. Our results demonstrate that the interaction between



temperature and herbicides is also related to the mode of action of the herbicides.

**WP014 Biomarkers in  $\alpha$ -cypermethrin-exposed *Daphnia schoedleri*:**

**Do they really correlate to demographic responses?** M. Arzate Cardenas, F. Martinez-Jeronimo, Escuela Nacional de Ciencias Biológicas-I.P.N. / Zoology. Synthetic pyrethroids are preferred over organophosphates and organochlorides because they are classified as moderately toxic to mammals and birds; nevertheless, it has been reported that they are highly toxic to aquatic organisms. Because cladocerans modify the energy content of their offspring according to the surrounding media to improve their odds in case they have to cope with stressful conditions at birth, this research was focused on the  $\alpha$ -cypermethrin (CYP) effect on *Daphnia schoedleri* energy allocation and demographic parameters (survival, fecundity, intrinsic rate of population growth, etc). Therefore, neonates (< 24 h) were exposed for 21 d to three sublethal concentrations of CYP: 0.54, 5.4 and 54 ng L<sup>-1</sup>, which correspond to 1/1000, 1/100 and 1/10 of the 48-h EC<sub>50</sub> previously determined. Partial Life Table Analysis was carried out with progeny and survival data. In addition, caloric content (CC) was calculated through the total quantification of protein, carbohydrates and lipids and then transformed to calories by using their combustion factor. CC used as a biomarker was expected to be the most sensitive response evaluated; nevertheless, demographic parameters such as survivorship and the net reproductive rate (R<sub>0</sub>) were more sensitive since they were significantly affected at the lowest concentration tested. Caloric content in progeny varied during the reproductive period assessed and could be related to fecundity as follows: the more neonates were born, the less energy could be provided by the adult females. These results suggest that stressed daphnids allocate more energy resources to their offspring, although this strategy can differ during among reproductive events and the surrounding conditions. Lastly, subindividual approaches should be accompanied by demographic studies which support predictions made through biomarkers assessment.

**WP015 Arachidonic acid partially reverses the deleterious reproductive effects of the juvenile hormone analog, pyriproxyfen** G.K. Gijupalli,

Clemson University / PHD student, Institute of Environmental Toxicology / PHD student; W.S. Baldwin, Institute of Environmental Toxicology / Department of Biological Sciences. The *Daphnia* genome project demonstrated the presence of a new nuclear receptor group (NR1L) that we named HR97 because of its similarity to the HR96 group involved in recognizing and responding to xenobiotics. HR97 is preferentially expressed in reproductive adults, and primarily present in the ovaries, gut, and mandibular area. Unlike HR96, the HR97 receptors are not promiscuous, and therefore are not toxicant receptors. Instead HR97 is only weakly activated by pyriproxyfen, but strongly inhibited by arachidonic acid (AA). No other activators or inhibitors have been found. Interestingly, arachidonic acid is the only n-6 polyunsaturated fatty acid that we tested that did not activate HR96. AA is one of only three fats that are sequestered in *Daphnia* (DHA, EPA), and AA is preferentially concentrated in the ovaries. Therefore, we hypothesized that AA may be involved in reproduction and possibly male production. We tested whether *Selenastrum*-based diets enriched with AA may enhance reproduction or repress male production induced by the juvenile hormone analog pesticide, pyriproxyfen. Acute and chronic toxicity tests demonstrate that AA has no significant effects on the number of offspring produced in the first and second generation at concentrations up to 4 mM. Modified chronic toxicity tests were performed in which 10-day old reproductively active *Daphnia magna* were exposed to 1.62 and 3.24 pM pyriproxyfen, or pyriproxyfen + AA (1 or 4 mM). AA had only minimal effects on male production, but significantly increased the number of females produced during pyriproxyfen co-exposures. Further studies examined the effects of other unsaturated fatty acids such as DHA, linoleic acid, and palmitic acid, in addition to AA on pyriproxyfen-induced changes in reproduction. Only AA significantly increased the female/male offspring ratio when co-exposed to pyriproxyfen. Exposure to only the fatty acids did not alter reproductive outcomes. The increase in female to male ratios in AA-pyriproxyfen co-exposure groups indicate that *Daphnia* use AA in reproduction and of the fats tested, only AA counteracts pyriproxyfen-mediated male production although the mechanism can only be estimated. Current studies are underway to examine how arachidonic acid enrichment effects male production when *Daphnia* are fed arachidonic acid poor diets (*chlorella*) or arachidonic acid rich diets (*Nannochloropsis*).

**WP016 Exposure-Dose-Response of *Saccostrea glomerata* (Sydney Rock Oyster) to cadmium obtained from suspended particulate material and phytoplankton** H.A. Schmitz,

University of Canberra / Institute of Applied Ecology; W.A. Maher, University of Canberra / Dept. of Health, Design & Science; A. Taylor, University of Canberra. Estuaries can receive anthropogenic contamination from both land and ocean sources making estuaries susceptible to anthropogenic pollution. However, the majority of anthropogenic marine pollution is land-based and originates from non-point sources such as from agricultural, commercial, and storm water run-off. The non-point sources of pollutants can transport metals into the environment. Metals are of concern because they can bioaccumulate to levels that may impair the health of organisms. The organism used for this research and is additionally a well-developed environmental biomonitor is the oyster. The *Saccostrea glomerata*, which are oysters endemic to Australia and New Zealand, are considered to be valuable biomonitors because they are: sedentary, easily accessible, and provide enough tissue for analysis. This research project will focus on cadmium, a potentially toxic metal that can come from various sources of pollutants. The *Saccostrea glomerata* were exposed to cadmium through suspended particulate material under controlled laboratory conditions. Separately, another experiment conducted was exposing the *Saccostrea glomerata* to cadmium-dosed phytoplankton under controlled laboratory conditions. For the first experiment the oysters were placed into microcosms with two dosages – approximately, one dose at 10 mg/kg and another dose at 50 mg/kg. For the second experiment the oysters were fed cadmium contaminated algae, *Chaetoceros muelleri*, with the dose of 1.3 X 10<sup>-8</sup> mol/l. During both experiments, the oysters were exposed to cadmium for 28 days. Afterwards, oyster tissues were collected for total metal analysis. Further biological measurements were taken; these measurements included total antioxidant capacity, lipid peroxidation and lysosomal destabilization. For the soil experiment a correlation between oyster tissue metal accumulation and lysosomal destabilization occurred. An increase in metal uptake incurred a higher level of lysosomal destabilization. Assessing all aspects of how the oyster responded to the cadmium as well as comparing both experiments' results will aid in determining the greater affect cadmium has on the oyster and the estuary ecosystem that it inhabits.

**WP017 Using a suite of biomarkers and bioindicators to assess the effects of complex contaminant mixtures on chronically exposed wild freshwater mussels** A.A. De souza machado,

Federal University of Rio Grande-FURG / Institute of Oceanography; C.M. Wood, McMaster University / Department of Biology; A. Bianchini, Universidade Federal do Rio Grande – FURG / Instituto de Ciências Biológicas; P.L. Gillis, Environment Canada / Aquatic Ecosystem Protection Research Division, Environment Canada / Aquatic Contaminants Research Division. Biomarkers and bioindicators are often used to identify contaminant-impacted aquatic ecosystems. In this context, biomarkers are early warning instruments, while bioindicators are ecological meaningful tools. However, there is a lack of information on how to integrate these groups of parameters and about how they respond to water chemistry. We evaluated the effect of water chemistry (metals, nutrients, pH, hardness, ammonia, etc.) on the responses of various bioindicators and biomarkers in wild *Lasimigona costata* along a gradient of complex urban-derived inputs. Bioindicators of general health including age, gonadosomatic index, relative growth rate, protein content, and condition factors were examined. Also, a suite of biomarkers were assessed in mussel gill tissues including metal bioaccumulation, tissue water content, major ions (Na, K, Ca, Mg) and oxidative stress responses (ROS, ACAP, SOD, CAT, GSH, SH protein, total SH, GR, GST, LPO). Water chemistry affected all groups of biomarkers examined ( $p < 0.0001$ ). We observed two similar-respondent groups: one related to urban proximity and other connected to waterborne metals. ROS, ACAP, SOD, CAT and some general health parameters likely reflected the proximity to urban centers. Close to urban centers mussels presented simultaneously increased LPO and decreased protein content and oxidant defenses. Tissue metal (Cu, Ni, Pb, Zn) concentrations were clearly related to waterborne metal levels ( $r = 0.98$ ). Bioaccumulation of some metals, such as Cu and Zn, were shown to be correlated ( $r = 0.65$ ). Tissue Na was not associated with metal bioaccumulation ( $r = -0.11$ ), while K, Mg and Ca were significantly related to metal accumulation ( $r = 0.73$ ). Although Ca granules were not measured, Ca tissue content increased comparably to metal tissue content demonstrating the protective role of Ca in metal detoxification. Mussels from the most metal-contaminated site also displayed the low condition factors and the lowest tissue water content. Moreover metal accumulation was related to oxidative stress



biomarkers ( $r=0.74$ ) and general health bioindicators ( $r=0.82$ ), indicating that general physiological disturbance may be the mechanism of multi-contaminant exposure-induced toxicity. Finally, these results demonstrate that complex environmental exposures can initiate detectable biomarkers at lower levels of biological organization (biomolecules, tissue damage) that are significantly related to whole-organism level impacts.

**WP018 Inhibition of feeding rate as a key mechanism of waterborne metal toxicity in bivalves** R. Chandurvelan, I. Marsden, University of Canterbury / School of Biological Sciences; S. Gaw, University of Canterbury / Department of Chemistry; C. Glover, University of Canterbury / School of Biological Sciences. The New Zealand green-lipped mussel, *Perna canaliculus* is a valuable aquaculture species, and also possesses characteristics suggesting that it is an ideal bioindicator organism for monitoring coastal metal contamination. To examine the responses of this species to waterborne cadmium (Cd), a multi-biomarker assessment was conducted examining impacts of acute (96 h; 2000 and 4000  $\mu\text{g Cd L}^{-1}$ ) and subchronic (28 d; 200 and 2000  $\mu\text{g Cd L}^{-1}$ ) laboratory exposures to waterborne Cd. A number of physiological (feeding or clearance rate, scope for growth), biochemical (metallothionein, glycogen, alkaline phosphatase activity), cellular and genotoxic (immune cell counts, occurrence of nuclear abnormalities) biomarkers were shown to be impacted by Cd exposure. Of all these biomarkers feeding rate was the most sensitive and responsive. Acute Cd exposure resulted in 78% (2000  $\mu\text{g L}^{-1}$ ) and 89% (4000  $\mu\text{g L}^{-1}$ ) lower feeding rates relative to control values. A decline in clearance rate was observed in mussels exposed to 2000  $\mu\text{g L}^{-1}$  after 5 days of subchronic exposure, and this effect strengthened and persisted throughout the course of the exposure, showing no evidence of recovery with time. Strong correlations between clearance rate and Cd accumulation in gill ( $R^2=0.805$ ) and digestive gland ( $R^2=0.875$ ) were observed on day 28 of subchronic exposure. Tissue accumulation was also correlated to Cd exposure level. The mechanism of feeding rate inhibition appeared to be behavioural, and mediated by valve closure. Importantly the decline in feeding rate also explained changes in other vital biological functions, suggesting that feeding inhibition has an indirect influence on many of the other key biomarkers. Field studies were conducted to determine if similar relationships between exposure and biomarker response were observed in mussels collected from polluted coastal settings. These studies also showed that impairment of feeding rate correlated well with tissue burden and environmental metal load. It is proposed that the decreased feeding rate of mussels has a significant impact on the overall physiological and biochemical status of mussels, and as such may be the key mechanism predicting individual, population and ecosystem health in near-coastal environments. The utility of feeding rate in mussels as a biomarker of exposure and effect is enhanced by the fact that it is easy to measure, can be performed rapidly at little cost and is amenable to field studies.

**WP019 Distinguishing between environmental and metal stress in wild yellow perch (*Perca flavescens*) using genomic, physiological and biometric indicators** J. Grasset, INRS / Centre Eau, Terre et Environnement; B. Bougas, INRS / Assessing Watershed Scale Impacts to Aquatic, Terrestrial, and Marine Ecosystems; E. Normandeau, Université Laval / IBIS; P.G. Campbell, INRS / Centre Eau, Terre et Environnement; L. Bernatchez, Université Laval / IBIS; P. Couture, INRS / Centre Eau Terre Environnement, INRS / Centre Eau, Terre et Environnement. Metal contamination is well known to induce metabolic stress in wild fish. However, several natural stressors also affect fish condition. Therefore, in order to detect metal stress in fish from contaminated environments, it is essential to also understand the effects of natural stressors on the condition of these fish. The global objective of this study is to understand the effects of non-metallic factors on yellow perch in order to help identifying biomarkers of metal stress that are both specific to individual metals and unaffected by natural stressors. In this project, we investigated the effects of three common natural stressors (heat stress, hypoxia and food restriction) in yellow perch (*Perca flavescens*) under controlled laboratory conditions, using genomic, enzymatic and biometric tools. Using a one-thousand gene cDNA microarray, we find that heat stress induced a difference in the transcription level of 388 genes, involved in particular in glycolysis, aerobic and anaerobic capacities, apoptosis and lipid metabolism. In contrast, hypoxia (75% oxygen saturation) and food restriction (25% of control feeding rate) had few effect on gene expression. However, all three stressors induced significant effects on the activity of pyruvate kinase (PK) and glucose-6-phosphate dehydrogenase (G6PDH) and on the condition factor (CF). These three stressors induced a decrease

in CF activity. Heat stress led to an increase in G6PDH activity whereas food restriction led to a decrease of the G6PDH activity. Hypoxia induced an increase in anaerobic capacities, as indicated by the augmented activities of PK and lactate dehydrogenase. Our data supports that natural stressors can affect growth rate, tissue metabolic capacities and energy accumulation in a similar fashion as metal stressors can. In a parallel study, the response of these endpoints to metal stress has also been characterized. Thus, our investigation will facilitate discriminating metal-induced stress from natural stress in wild yellow perch. Finally, the relationships between the genomic and the physiological endpoints will also be discussed.

**WP020 Exploratory Analyses of Approaches for Characterizing Major Ion Toxicity (MIT)** P.R. Paquin, HDR|HydroQual; D.R. Mount, US Environmental Protection Agency / ORD; C. Fanelli, HDR | HydroQual; R. Hockett, US EPA / ORD; C. Delos, US Environmental Protection Agency, U.S. EPA / Mail Code 4304, US Environmental Protection Agency; W. Colon-Jusino, US EPA. Significant advances in understanding have been made in recent years in regard to how trace metals (e.g., Ag, Cd, Cu, Pb and Zn) exert toxicity and how to predict aquatic organism effect levels over a range of water quality characteristics. Underpinning this approach has been an integration of geochemical, physiological, and toxicological information, leading to a robust predictive framework. While considerable attention has also been directed at investigating the toxicity of major ions (e.g., Na, K, Ca, Mg, Cl,  $\text{SO}_4$ ,  $\text{HCO}_3$ ), there has not yet been an approach developed that provides a similar integration of information into a predictive modeling framework for major ions. The situation is further complicated by the fact that many natural waters contain elevated levels of several major ions, creating the need for approaches that explicitly or implicitly account for the effects, toxic or ameliorative, of multiple ions. One approach has been to use multivariate regression modeling to establish exposure – effect relationships for individual species exposed to well-characterized mixtures of major ions under lab conditions. Some approaches have used aggregate measures of exposure such as conductivity, salinity, total dissolved solids, often with restrictions on the range of ion composition considered. Others have focused on the physiological responses of the organism (e.g., changes in trans-epithelial potential) to better understand abiotic-biotic interaction from a more mechanistic perspective. This presentation will introduce examples of these alternative approaches, in combination with results of exploratory data analyses, in an effort to begin to identify and formulate a unified, broadly applicable approach to evaluating MIT.

**WP021 Application of Quantitative Proteomics to Evaluate Effects of Wastewater-impacted Coastal Stormwater on the Fathead Minnow (*Pimephales promelas*)** K.J. Ralston-Hooper, Visiting Dupont Research Scientist at Duke University / Nicholas School of the Environment, DuPont / Haskell Global Centers; G.J. Getzinger, Duke University / Nicholas School of the Environment; M.E. Turner, E.J. Soderblom, A. Moseley, Duke University School of Medicine / Proteomics Core Facility; A.S. Kolok, University of Nebraska Medical Center / Environmental, Agricultural and Occupational Health, University of Nebraska – Omaha / Nebraska Watershed Network, University of Nebraska – Omaha / Department of Biology; R. Hoke, DuPont, Haskell Global Centers, E.I. DuPont de Nemours and Co., E.I. DuPont de Nemours and Co. / Haskell Global Centers for Health and Environment; L. Ferguson, Duke University / Department of Civil and Environmental Engineering, Duke University / Nicholas School of the Environment, Duke University / Department of Civil & Environmental Engineering, Pratt School of Engineering / Department of Civil & Environmental Engineering. Environmental impacts associated with extensively managed turfgrass areas include increased pesticide, fertilizer, and water use. The use of reclaimed municipal wastewater for golf course irrigation has been implemented to help mitigate water use issues; however, recycled wastewater may contain pathogens, viruses, bacteria, trace organics, heavy metals, and endocrine disrupting compounds that could pose risks to human and environmental health. We have conducted field exposures to evaluate the effects of reclaimed wastewater irrigation on aquatic biota in coastal stormwater detention ponds associated with golf courses on Kiawah Island, SC. Adult male fathead minnows (*Pimephales promelas*) (FHM) were exposed to stormwater at 3 individual detention pond locations as well as to treated wastewater at the Kiawah Island Utility WWTP and to dechlorinated tap water (control). After 14 days, FHM were euthanized and tissues collected (brain, liver, gonad, plasma). Label-free, non-gel proteomic analyses were performed on FHM liver, brain, and gonad tissues from the exposed

fish, and 1084, 1023, and 60 proteins were identified in tissues respectively. Many of the differentially-expressed hepatic proteins in the treatment groups were linked to possible endocrine disruption and/or immune responses. Estrogen receptor was up-regulated in FHM exposed at the WWTP as well as other proteins associated with immune responses such as angiotensinogen, sialoadhesin, and malectin. Differentially-expressed proteins in brain tissues also were consistent with endocrine and/or immune responses. For example, sex hormone binding globulin and lysosomal protective proteins were both up-regulated in brain tissues of WWTP effluent-exposed FHM when compared to controls. One-third of the 60 gonadal proteins identified were histone and ribosomal proteins. Heat shock protein 90 kDa and tropomyosin isoform 2 were the only two gonadal proteins expressed across all treatment groups. When differentially-expressed proteins were analyzed by Ingenuity Pathway Analysis (IPA), Antigen Presentation, Humoral Immune Response, Inflammatory Response was one of the top impacted networks. Overall, proteomic analysis allowed elucidation of endocrine effects as well as previously unreported immunotoxic effects in FHM following exposure to stormwater and wastewater associated with wastewater reuse practices on Kiawah Island, SC.

**WP023 Reversible Interference of Gill Function in Fishes Following Exposure to the Lampricide 3-Trifluoromethyl-4-nitrophenol (TFM)** L.A. Sorensen, Wilfrid Laurier University / Department of Biology; J. Wilson, Centro Interdisciplinar de Investigaç o Marinhae Ambiental (CIIMAR); M.P. Wilkie, Wilfrid Laurier University. The lampricide 3-trifluoromethyl-4-nitrophenol (TFM) is widely used in the Great Lakes to control populations of invasive sea lampreys (*Petromyzon marinus*). This pesticide targets larval lamprey by uncoupling oxidative phosphorylation in the mitochondria, which impairs ATP production. It also has been reported to impact the gills of lamprey and non-target fishes. For these reasons, we hypothesized that TFM would interfere with gill ATP-dependent ion uptake by the fresh water trout, a non-target species. Accordingly, we measured Na<sup>+</sup>, K<sup>+</sup>-ATPase (NKA) and vacuolar H<sup>+</sup>-ATPase activity, protein levels (Western blot) and immunohistochemical localization in addition to plasma Na<sup>+</sup> and Cl<sup>-</sup> concentration in TFM-exposed juvenile rainbow trout (*Oncorhynchus mykiss*) acclimated to hard water (Hardness ~ 200 mg l<sup>-1</sup> CaCO<sub>3</sub>). Rainbow trout were exposed to 7.6 mg l<sup>-1</sup> TFM for 0, 3, 6, 9-h, which was equivalent to the 12-h LC99.9 of larval lamprey and representative of a dose they could inadvertently encounter in the field. Gill function was also tracked following a 12-h exposure and 24-h recovery period. By 9-h, exposure to TFM caused a significant drop in plasma Na<sup>+</sup> and Cl<sup>-</sup> concentration of 4% and 6%, respectively. Following depuration in TFM-free water, plasma Na<sup>+</sup> concentration increased by 7% as compared to the 9-h TFM exposure group and no significant change was found for chloride. Decreases may have been compensated for by an initial transient increase in NKA activity at 3-h, which decreased to pre-exposure values thereafter, where it remained through to 24-h of depuration. H<sup>+</sup>-ATPase was sustained during the course of the experiment and no significant differences were observed with TFM exposure time. Although TFM is thought to damage the gills in non-target fishes, these results suggest that the effects of TFM on gill-mediated ion-exchange are mild and reversible. Currently, immunohistochemistry and western blotting is being used to quantify and localized branchial NKA and H-ATPase following TFM exposure.

**WP024 Alterations in reproductive capacity, oxidative stress and enzymatic biomarkers in an anadromous fish: natural variation or contaminant exposure?** P. McClellan-Green, North Carolina State University / Ctr for Marine Sciences & Technol; W. Green, Davidson College; L. Boddiford, Duke University Marine Laboratory; E. LaRoque, Barton College. The spawning stock biomass of blueback river herring is declining and juvenile production remains low. Causes for the decline are unknown but theories of why this is occurring range from over harvesting of the population to anthropogenic impacts from contaminants and poor water quality. We recently initiated a study examining the levels of oxidative stress and the expression of biomarkers of pollution in blueback river herring from the Chowan River, NC. Our goal was to compare/correlate these results with the reproductive capacity of herring caught throughout their spawning season. Blueback herring were collected weekly, for a total of 8 consecutive weeks, from a single site during the spawning runs in the spring of 2010 and 2011. Physical parameters such as length, weight, age, spermatocrit/egg number and VTG levels were measured. Blood, liver and gonad tissue were collected from each animal. Analysis of reproductive parameters indicate

their breeding potential is highest during the initial three weeks of spawning and decreases as the season progresses. Blood levels of 11-ketotestosterone, testosterone and estradiol were determined and compared to reproductive status. Oxidative stress parameters were measured in liver tissue. Total glutathione levels were highest during week 4 in male bluebacks and weeks 4 and 5 in female bluebacks. LPO was highest in bluebacks of both genders during the initial weeks of spawning and decreased as spawning progressed. The results indicate that overall oxidative stress increases up to the peak spawning period and then declines as spawning ends. Our next step was to analyze cytochrome P450 levels (including P450 1A) using enzyme activity and western blots. Results for these latter assays are pending. This study attempts to delineate changes that occur in various reproductive parameters, the oxidative stress response and the cytochrome P450 monooxygenase system during spawning. Our ultimate goal is to determine which changes are due to natural spawning variation and which are produced following pollutant exposure. These results could have distinct implications for monitoring during field studies.

**WP025 How to identify the metabolic mechanism of action (mMoA) from toxicity data? Comparing species and effects in a Dynamic Energy Budget (DEB) framework** E.I. Zimmer, Vrije Universiteit / Department of Theoretical Biology; S. Augustine, IRSN / Laboratory of Radionuclide Ecology; T. Jager, S.A. Kooijman, Vrije Universiteit. Mathematical modeling in ecotoxicology contributes to understanding effects of toxicants on individuals and allows extrapolating these effects from laboratory to field conditions, from individuals to populations and across species of organism and type of compounds. The OECD guidelines for the testing of chemicals represent a large collection of testing methods used to assess the effects of chemicals on organisms on a routine basis. The analysis of such effects would benefit from a unified modeling framework. Dynamic Energy Budget (DEB) theory provides a conceptual framework that explains how organisms allocate energy from food to growth, reproduction, maintenance and development. The same framework can be applied to all organisms (micro-organisms, plants, animals) and on all levels of biological organization. Parameter values are species-specific, while the intra-species variation is typically small. Effects of toxicants translate into changes in one or more parameter values; the affected parameter defines the metabolic mechanism of action (mMoA). For each mMoA, a specific combination of effects on measurable endpoints exists, e.g. effects on growth, reproduction, development, feeding, and respiration. Although several studies have shown the potential of understanding toxic effects in a DEB framework, standard toxicity data usually do not contain sufficient information to accurately determine the mMoA. Some mechanisms show similar patterns of effect under standard test conditions, so that uncertainties remain. Simple extra measurements can solve this problem, however, that hardly affect the financial costs of testing. The aim of this study is to determine the minimum combination of endpoints which need to be assessed to identify each possible mechanism of action. This will facilitate experimental planning and enhance interpretation of test results. To this end, we simulated all possible mMoAs in several aquatic species (e.g. the OECD standard test organisms *Daphnia magna*, *Danio rerio* and *Pimephales promelas*) and illustrate which endpoints theoretically identify the mMoA. We demonstrate that knowledge of mMoAs is essential for extrapolation to the population level.

**WP026 Effects of the Pesticides Atrazine, Linuron, and Vinclozolin on Steroidogenesis in the Amphibian, *Xenopus tropicalis*** D.L. Gordon, Canadian Rivers Institute, University of Prince Edward Island / Department of Biology; M.R. Van den Heuvel, Canadian Rivers Institute, University of Prince Edward Island; N.S. Hogan, University of Saskatchewan / Toxicology Centre. Agriculture chemicals such as pesticides have been a major concern in the normal reproduction and development of amphibians. However, there are few studies that have assessed the potential for these compounds to directly impact functional responses such as steroidogenesis and thus shed light on potential mechanisms of action underlying higher-level reproductive effects. Therefore, the aim of this study was to determine whether the pesticides atrazine, linuron, and vinclozolin are able to disrupt steroidogenesis and whether this response is mediated through changes in expression of steroidogenic genes in the amphibian, *Xenopus tropicalis*. An *ex vivo* protocol for determining steroid production, and measuring the expression of target genes in ovarian tissue was optimized through a time course experiment with tissues exposed to the stimulator of steroidogenesis, human chorionic gonadotropin (hCG) for 2, 4, 8, and 16 h and it was determined that an

optimal incubation period to measure both endpoints was 8 h. The impact of each pesticide on steroidogenesis was evaluated following incubation with 0.1, 1 and 10 mg/mL for 8 h. Atrazine caused an increase in testosterone and a decrease in estradiol production. A decrease in testosterone was observed after incubation with linuron and vinclozolin, and linuron also decreased estradiol levels. An increase in STAR mRNA following linuron exposure was the only change in transcript levels. The results demonstrate that the *ex vivo* steroidogenesis bioassay is an effective tool to determine the potential for these pesticides to alter steroid biosynthesis, but that changes in hormone production may not be mediated through changes in levels of steroidogenic genes.

**WP027 Effects of TDCPP and TPP on reproduction and hypothalamus-pituitary-gonad axis in adult zebrafish (*Danio rerio*)** X. Liu, Seoul National University, Seoul National University / School of Public Health; K. Ji, University of Saskatchewan / Biomedical Veterinary Sciences and Toxicology Centre, University of Saskatchewan; K. Choi, Seoul National University / School of Public Health. Tris(1,3-dichloro-2-propyl) phosphate (TDCPP) and triphenyl phosphate (TPP) belong to a group of chemicals collectively known as triester organophosphate flame retardants (OPFRs). OPFRs are used in a wide range of consumer products and have been detected in biota, including free-living avian species; however, data on toxicological and molecular effects of exposure are limited. In present study, paired adult male and female zebrafish (*Danio rerio*) were exposed to various concentrations of TDCPP and TPP for 21 days, and the effects on reproduction, condition factor (CF), gonadosomatic index (GSI) and hepatosomatic index (HSI) were evaluated. In addition effects on plasma vitellogenin (VTG) and related gene transcription along the hypothalamic-pituitary-gonad (HPG) axis were checked after exposure. The results showed that plasma VTG level was significantly increased and mean fecundity rates were significantly decreased by exposure to TDCPP and TPP. No significant changes were observed in CF, GSI or HSI in both females and males. The gene transcription profile along HPG axis was sex-dependent. In males, up-regulation of *GnRH2*, *GnRHR3*, *CYP19B*, *ERα*, and *ER2β1* in brain, and *FSHβ* in pituitary, and down-regulation of *LHβ* and *AR* in brain were observed. Corresponding to the up-regulation of *FSHβ* and down-regulation of *LHβ* in brain, *FSHR* was up-regulated and *LHR* was down-regulated in testis. Among steroidogenesis genes, transcriptions of *HMGR*, *StAR* and *17βHSD* were down-regulated, while those of *CYP11A*, *CYP17* and *CYP19A* were up-regulated. In females, down-regulation of *GnRH2* and *GnRHR3*, and up-regulation of *FSHβ*, *LHβ*, *CYP19B*, *ERα*, *ER2β1* and *AR* were found in brain. In ovary, *FSHR* and *LHR* were significantly up-regulated. Except for *CYP11A*, all other steroidogenic genes were significantly up-regulated. The observed changes of GnRH and gonadotropins can be further related to the subsequent disruption in both sex steroid hormone balance and plasma VTG levels. Potential implication in reproduction performance warrants further study.

**WP028 Sublethal Exposure to Vinclozolin Modulates Gonad Development and Impairs Reproduction in Zebrafish** E. Hicks, J. Weigand, S. Grandstrand, A. Revak, T. King Heiden, University of Wisconsin – La Crosse / Department of Biology. Exposure to endocrine disruptors during critical periods of gonad development can modulate or functionally reverse sexual differentiation of fish, and can also impair development or maturation of the gonad. These alterations can adversely impact the sustainability of feral fish populations by influencing sex ratios and impairing reproduction. We are using the zebrafish as a model to better understand the potential impacts of developmental exposure to anti-androgenic compounds on fish reproductive capacity. Zebrafish that were exposed to sublethal concentrations vinclozolin (400 µg/l) during the period of sex differentiation had altered gonad development, and sex ratios were shifted towards females. Upon maturation, females showed precocious ovarian development, while testis development was inhibited. Physiological responses emulated histopathology in that egg release was not greatly impacted, but male fertility was reduced. Interestingly, impacts on the gonad do not appear to be permanent, suggesting impaired fertility could result from alterations in spawn behaviors or impaired sperm quality. We are currently testing these hypotheses to establish physiological responses for directing mechanistic studies.

**WP029 Benzo(a)pyrene exposure effects on reproductive success, sexual development, gene expression and DNMT enzyme activity in zebrafish** C. Thornton, University of Mississippi / Department of Pharmacology and Environmental Toxicology Research Program, University of Mississippi

/ Pharmacology; J. Corrales, University of Mississippi / Environmental Toxicology; X. Fang, University of Mississippi; M. White, K. Mislan, K.L. Willett, University of Mississippi / Environmental Toxicology Research Program. Benzo(a)pyrene (BaP) is an environmentally relevant carcinogenic and endocrine disrupting compound that causes multigenerational effects in mammals. Polycyclic aromatic hydrocarbons (PAHs), like BaP, are implicated in preterm deliveries, low birth weights, and childhood cancers in offspring of exposed mothers. We hypothesized that BaP exposure will adversely affect reproduction, alter gene expression, and cause quantifiable pathologies. Zebrafish embryos/larvae whose parents had been exposed (N=3 tanks per treatment) were continuously exposed to waterborne BaP (42 µg/L) until 96 hpf. Reproductive success was impaired in the parental generation and histopathological alterations, such as heart deformities, ocular defects, and spinal curvature, were non-significantly increased and hatching was delayed in the BaP exposed larvae. Larval global DNA methylation, but not DNMT enzyme activity, was reduced by BaP. To distinguish whether effects were due to (1) parental exposure or (2) larval exposure alone two additional studies were designed. In experiment 1, adult zebrafish (2 females x 2 males, N=10 replicate tanks per treatment) were fed 2%BW/day flake food treated with 0, 11.6, 110, 1086 µg BaP/g flake (equivalent to 0, 0.23, 2.2, and 22 µg BaP/g fish/day). Reproductive success, survival, and morphological abnormalities of offspring were measured. Larvae were collected for pathology to determine whether BaP caused an effect on gonadal differentiation at various time points throughout development (20, 30 or 120 dpf). Methylation status and DNMT activity were measured in liver and gonad. In experiment 2, zebrafish larvae whose parents were never exposed were fed BaP-treated Artemac during gonad differentiation (10-20 days post fertilization (dpf; ovary development) or 21-30 dpf (testes development)). The same endpoints were measured for experiment (2) as in experiment (1). Based on these results, BaP has the potential to negatively impact zebrafish reproduction as well as their offspring's development. Supported by NIEHS R21ES019940.

**WP030 Assessing the effects of phenanthrene on ovarian tissue of fathead minnow (*Pimephales promelas*) using both in vivo and in vitro bioassays** J.R. Loughery, University of New Brunswick / Biology; N. Chiasson, University of New Brunswick; R. Wood, A. Mercer, K. Kidd, C. Martyniuk, University of New Brunswick / Biology. Phenanthrene (PHEN), a polycyclic aromatic hydrocarbon (PAH), is found in aquatic systems around urban environments and oil extraction facilities. While stress responses induced by PAHs in teleosts have been extensively studied, recent studies suggest some PAHs, including PHEN, may also be endocrine disrupting substances (EDS). To explore potential effects of PHEN on the reproductive axis, explants of ovarian tissue of fathead minnows (FHM; *Pimephales promelas*) were exposed *in vitro* to three nominal concentrations of PHEN ( $10^{-6}$  M,  $10^{-8}$  M and  $10^{-10}$  M; corresponding to 0.18 ppm, 1.78 ppb and 17.82 ppt) over a 12 hr period. PHEN did not significantly affect estradiol ( $E_2$ ) production or the mRNA levels of steroid-related genes that included aromatase,  $17\beta$ -hydroxysteroid dehydrogenase, estrogen receptors, or genes in the aryl hydrocarbon pathway. FHM were also exposed *in vivo* for up to 72 hrs to waterborne PHEN at target concentrations of 0, 50 and 500 ppb (environmentally relevant) and a high concentration of 5000 ppb. Fish were sacrificed after 24, 48 and 72 hrs. Statistical analysis of morphometric data across concentrations, taking into account time points, suggested effects to GSI and LSI after 72 hr exposure. Gene expression analyses are presently being conducted on ovary from the 48hr *in vivo* time point to correlate to the morphological changes after 72 hr. These data suggest that that PHEN does not affect  $E_2$  production or genes in the steroid pathway using a short term *in vitro* exposure but that this PAH can disrupt higher level endpoints in the FHM *in vivo* at higher concentrations.

**WP031 Protein Expression in the Cortisol-Producing Interrenal Tissues in Endocrine Disrupted Fish from Southern California's Urban Ocean** D.R. Causey, California State University, Long Beach; J.A. Reyes, Pacific Coast Environmental Conservancy; C.M. Waggoner, A.W. Hamilton, California State University, Long Beach; J.L. Armstrong, Orange County Sanitation District / Ocean Monitoring Division; K.M. Kelley, California State University, Long Beach. Discharge locations of wastewater treatment plants (WWTPs) located on the coast of southern California have been previously shown to be associated with physiological effects in wild fish. In recent work, it has become evident that an impairment in the production of the stress and metabolic hormone, cortisol, is correlated with exposures to



environmental contaminants. This form of endocrine disruption is observed as an inability to activate a normal neuroendocrine response to stress and has been detected in several species, including English sole, hornyhead turbot, and California scorpionfish. When interrenal tissue samples from English sole were subjected to an *in vitro* ACTH challenge, it was found that individuals sampled from WWTP discharge areas had impaired cortisol responses. Furthermore, their inability to produce cortisol was significantly related to a corresponding drop in expression of key mRNAs in the interrenal, including for steroidogenesis-activation regulator (StAR) and P450-11 $\beta$  hydroxylase. A proteomics screening approach is also being used to determine alterations in interrenal protein expression associated with this form of endocrine disruption. These emerging data will be discussed as part of the effort to elucidate the molecular mechanisms underlying disruptions in interrenal functions. (Support from NOAA/USC Sea Grant Program in California).

**WP032 Integrated Remediation and Ecological Restoration: A Case Study in Woodbridge, New Jersey** M. Parkes, M. Laska, E. DeCelles, Great Ecology. The Former Nuodex Corporation facility is a 185-acre site located in Woodbridge Township, New Jersey operated as an organic chemical manufacturing facility for much of the last century. Historically, much of the site was tidal wetlands, but approximately two thirds of the site was filled via dredge material placement during channel deepening of the Raritan River during the 1940's and 1950's. This converted the tidal wetlands to stormwater-fed, freshwater wetlands mainly vegetated by an invasive plant, *Phragmites australis*. The site became contaminated with a suite of constituents including Dense Non-aqueous Phase Liquids (DNAPLs) in groundwater as a result of the facility's operation. DNAPL in groundwater can be problematic to delineate and remediate. In 2008 Great Ecology and Brown and Caldwell initiated the remedial and ecological investigation for the current site owner, EPEC Polymers Inc. The subsequent remedial action workplan included a hydraulic barrier wall to contain groundwater impacted by DNAPL source material that resulted in approximately 30 acres of permanent impacts to open water and wetland. An integrated approach to the remediation and required wetland mitigation was necessary to achieve the project goals of attaining cleanup standards protective of human health and the environment, compensating for wetland impacts due to implementation of the remediation, preparing the site for eventual redevelopment, and providing public access to the Raritan River in Woodbridge Township for the first time in over 100 years. We present our innovative approach to remediation, wetland mitigation, regulatory compliance, and stakeholder outreach. This strategic approach resulted in a novel, integrated design now under construction.

**WP033 An Integrated Ecology and Design Approach to Wetland Restoration** M.S. Laska, Great Ecology, Great Eastern Ecology, Inc.. Habitat restoration projects on remedial, contaminated, and/or Brownfield sites are driven by environmental regulations (e.g., CERCLA, RCRA, CWA), presenting opportunities for creative approaches to regulatory compliance through beneficial site reuse and optimization of habitat/wetland restoration design. Numerous sites never achieve full potential, often because there is little opportunity for involvement by ecologists, designers, and landscape architects until a relatively late stage in the restoration process (if at all). Some pioneering projects, however, have integrated ecological and design perspectives at an early stage – with more predictable and highly favorable financial, public, environmental and ecological outcomes. These projects highlight the innovative, cost effective, and sustainable solutions that result from linking ecology and design perspectives to sites historically lead by remedial engineers. Our talk and this symposium explore a more ecologically oriented approach to habitat and wetland restoration; discuss where and when our approach adds value to the restoration ecology of a habitat; and present successful case studies for a range of restoration designs in several challenging locations.

**WP036 Restoration of Estuarine Salt Marsh Impacted by a Subsurface Petroleum Hydrocarbon Release on the East Coast of the USA** C. Pfeifer, Cardno Entrix. Restoration of hydrocarbon-impacted salt marsh at the site of a subsurface release of No. 2 fuel oil illustrates the additional complexities and uncertainties that often distinguish restoration of contaminated wetlands from similar efforts in more pristine settings. Salt marsh and shallow water habitats were exposed to dissolved and free-phase petroleum hydrocarbons when LNAPL from a leaking aboveground storage tank at an industrial

facility daylighted along the shoreline of a tidal estuary. *In-situ* shoreline remediation was favored by regulators over more invasive approaches. After remedial goals based on product thickness were achieved, efforts to restore the affected wetland habitat and ecological services were undertaken as part of a natural resource damage assessment (NRDA). Prior to initiating primary restoration, a screening-level risk assessment was performed to confirm site suitability for transplanting appropriate species of native salt marsh vegetation. While residual poly-aromatic hydrocarbons (PAHs) in intertidal sediments were elevated compared to background, concentrations remained below those measured at successfully restored salt marsh sites reported in the literature. However, within the first growing season *Spartina alterniflora* transplanted directly into remediated sediments experienced significant mortality. Subsequent investigations revealed recontamination of the shoreline due to decreasing effectiveness of upgradient remedial controls through time. The nature and extent of shoreline and nearshore sediment contamination was reevaluated using ecological criteria based on equilibrium partitioning and toxicity to benthic invertebrates. Sediment removal and replacement was selected as the preferred option for achieving revised remedial goals based on ecological risk. Approximately 500 cubic yards of hydrocarbon-impacted sediment was excavated and replaced with clean sand. Salt marsh vegetation was again transplanted throughout the remediated shoreline. In addition, pre-vegetated coconut fiber mats were installed along the leading edge to provide immediate stabilization and erosion control. Performance standards established for post-construction monitoring included risk-based metrics for sediment quality and benthic community health in addition to more typical criteria based on vegetation parameters. Habitat restoration was ultimately successful.

**WP037 An ecological approach to restoration of a PCB-contaminated pond** M. Peterson, Oak Ridge National Laboratory; M. Ryon, Oak Ridge National Laboratory / Department of Environmental Sciences; K. Roy, Oak Ridge National Laboratory / Environmental Sciences Division; J.G. Smith, ORNL / UT-Battelle / Environmental Sciences Division; T. Mathews, Oak Ridge National Laboratory / Environmental Sciences Division, Oak Ridge National Laboratory / Biological and Environmental Sciences Division. The 25-acre P1 Pond, located on the Oak Ridge Reservation in East Tennessee, received inputs of polychlorinated biphenyls (PCBs) from industrial operations for decades, resulting in contaminated water, sediments, and biota. Largemouth bass (*Micropterus salmoides*) in this pond, prior to remedial actions, had PCB concentrations >10-fold above state and federal guidelines for assessing human health concerns. Rather than using conventional remediation strategies such as dredging the contaminated sediments, an ecological approach to remediation was adopted. The pond was altered by: 1) drawing down the water and capping contaminated sediments with clean fill, 2) planting native, rooted, aquatic macrophytes such as pickerel weed (*Pontederia chordata*) and American lotus (*Nelumbo lutea*) to further stabilize contaminated sediments, 3) removing invasive and upper trophic level fish species which disturb contaminated sediments and accumulate high concentrations of PCBs, and 4) restocking the pond with smaller, short-lived native fish (minnows, sunfish, etc.) which have less of a tendency to accumulate PCBs. Monitoring results have shown that in the three years since remediation was initiated: 1) growing season macrophyte colonization has expanded to approximately 40% of the pond's surface, 2) the fish community structure changed from a shad/sunfish/bass-dominated system to a sunfish-dominated system, and 3) PCB concentrations in resident fish have decreased by 50%.

**WP038 Preservation state of a natural wetland to propose the integrated management** E.V. Perez, Universidad Autonoma de San Luis Potosi / Programa Multidisciplinario de Posgrado en Ciencias Ambientales, / Universidad Autonoma de San Luis Potosi; M.C. Alfaro, Universidad Autonoma de San Luis Potosi / Facultad de Ciencias Quimicas; R. Briones, Universidad Autonoma de San Luis Potosi / Instituto de Metalurgia; P. Medellin, Universidad Autonoma de San Luis Potosi / Agenda Ambiental. There are few inland wetlands in Central Mexico due to the perturbations caused by the sugar cane culture. Only two wetlands remain in the Mexican state of San Luis Potosi and are considered as a RAMSAR sites. However they are exposed to a several stress conditions caused by rural towns, agriculture, livestock, and the changing conditions of precipitation caused by the climate variability that could affect their trophic. In this work, the contribution of the sediments with nutrients and the discharge of groundwater are studied to estimate the availability of nutrients in the wetland "Cienega de Tamasopo, SLP, México; RAMSAR site (1814)". The experimental approach has

been monitoring of P, Fe, S, Ca in porewater in five sites in the wetland, also, bottom sediments collected at two sites in the wetland have been tested in experimental columns under controlled conditions to evaluate the effect of pH and pE changes, the input of Fe, phosphates and Ca, on the P availability to hold or increase the wetland productivity. The pH and redox potential affect the mobility of P and Fe at the sediment-water interface; the Fe mobility is related to the redox behavior of S. Our results suggest that dissolved phosphate is depleted and its chemical behaviour is related to the Fe and S cycles. It seems that the main chemical processes which determine the mobility and availability of the  $P-PO_4$ , are associated with the dissolution-precipitation reactions (Ca, Fe) and the reducing conditions that affect the Fe,  $S^{2-}$  and  $SO_4^{2-}$  species. Also, the results in the sediments indicate nutrient enrichment (P, Fe, Ca) probably related to the use or recycling of nutrients during the plants productivity and decomposition in the sediment. The porewater results indicate P enrichment at sites where agriculture is important and thus we assume that agriculture has alliterated the trophic state of the wetland. If this is the case, a management plan should be implemented with strategies focused to retard the degradation of the wetland resources. The management plan needs to be consented with users of this ecosystem because they do not have the knowledge of its values and the awareness about the importance of the intervention activities that could affect them.

**WP039 Assessing Changes in Contaminant Fluxes Following Dam Removal in an Urbanized River** M.G Cantwell, / Atlantic Ecology Division; M.M. Perron, NRC/EPA; J. Sullivan, US EPA; D.R. Katz, US EPA / Atlantic Ecology Division, U.S. EPA / ORD\NHEERL\AED. Restoration of rivers and their associated ecosystems is a growing priority for government agencies (e.g., NOAA, USEPA), as well as conservation organizations. Dam removal is a major component of many restoration projects credited with reintroducing fish species, improving water and habitat quality, and increasing recreation potential. Many proposed locations for restoration, however, have been impacted by past discharges of chemical pollutants. As a result, the potential exists for the release of sequestered contaminants following dam removal or other restoration activities. Few, if any, dam removal projects have studied the changes in contaminant fluxes following removal. In this study, we measured dissolved and particulate concentrations of polynuclear aromatic hydrocarbon (PAHs) and polychlorinated biphenyls (PCBs) before and after removal of an existing low head dam in the Pawtuxet River, an urbanized river in Cranston, RI. Preliminary results indicate that dissolved concentrations of PAHs decreased at one site above the dam location following removal, while concentrations remained unchanged below the dam at the mouth of the river which discharges to Narragansett Bay. Dissolved concentrations of individual PCB congeners were very low overall, remaining below  $1 \text{ ng L}^{-1}$  at all sites. At all sites, total dissolved PCB concentrations were lower in the second post-removal sampler deployment compared to before dam removal. Total particulate concentrations of PAHs and PCBs collected in sediment traps showed a decrease following dam removal at all sites. Results from this study will be used to evaluate methods under development to assess the short and long-term impact of ecological restoration activities such as dam removal.

**WP040 Alumnae Valley Landscape Restoration: Responding to Environmental Toxicology through the Design of the Built Environment** R. Gleeson, L. Solano, M. Urbanski, M. Van Valkenburgh, Michael Van Valkenburgh Associates, Inc., Landscape Architects. The original plan for Alumnae Valley, a 13 acre site at Wellesley College, in Wellesley, Massachusetts, had been to restore the experiential potential of the site. This was to be achieved by replacing a parking lot with a naturalized landscape linking a new student center to Lake Waban. As a means of improving water quality within the larger watershed, the plan was to restore the site's hydrological functions of recharging groundwater and filtering storm drainage flowing to Lake Waban. During the course of the design, geotechnical and environmental testing revealed severe soil toxicity issues stemming from earlier uses of the site as the location of a coal gasification plant, a landfill, and underground oil tanks that served the central utility plant. As plans to reestablish this area as an important new wetland landscape evolved, these overlapping eras of contamination required that restoration of the Valley employ multiple remediation strategies, including isolating toxic soils and safely reusing them on site and establishing a long term pumping system to draw out dense, non-aqueous phase liquid that had settled in the site's original peat layer. The innovative technology employed in the restoration of Alumnae Valley was a necessary component in the college's aspiration to disrupt what

had become a pattern of an increasingly unbalanced relationship between the human habitation of the site and its ecological functions. Although the soil pollution and stormwater treatment were separate issues, the landscape design adopts an integrated approach to addressing them simultaneously. The complex restructuring was driven by the technical requirements of the ongoing remediation operations also created the conditions whereby a "restored" site hydrology could become a prominent theme in the landscape experience. The methodology for integrating the treatment of soil contaminants, restoration of wetlands and site hydrology, and improvements to the social enjoyment of the landscape will be presented, along with related examples of wetland restoration on post-industrial sites.

**WP041 A chronic Pb Biotic Ligand Model for *Ceriodaphnia dubia*** K.A. De Schamphelaere, Ghent University (UGent) / Environmental Toxicology and Aquatic Ecology, Ghent University / Environmental Toxicology and Aquatic Ecology; C. Nys, C. Janssen, UGent. We investigated the individual effects of pH and Ca on the chronic toxicity of  $Pb^{2+}$  on *Ceriodaphnia dubia* using 7 days chronic reproduction tests. The data showed no influence of Ca on chronic Pb toxicity, while the effect of pH could be described by a conventional BLM-type competition between  $H^+$  and  $Pb^{2+}$  ions. A Biotic Ligand Model (BLM) was developed that incorporated this effect as well as the effect of DOC on Pb complexation, using the NICA-Donnan formulation implemented in Visual Minteq. This BLM was then successfully validated with four independent chronic toxicity datasets comprising a range of synthetic and natural waters. This potentially allows the application of a chronic Pb-BLM for deriving bioavailability based Pb water quality criteria in Europe, USA, and elsewhere.

**WP042 Accumulation and toxicity of selenium in fish associated with mountaintop removal coal mining effluent** M.C Arnold, Duke University / Nicholas School of the Environment, Duke University; T. Lindberg, Duke University / Nicholas School of the Environment; Y. Liu, H. Hsu-Kim, Duke University / Pratt School of Engineering; R. Di Giulio, Duke University / Nicholas School of the Environment. Water quality in the main stem of the Mud River in West Virginia is significantly impacted by mountain top removal (MTR) coal mining activities. One of the major contaminants of concern in this 14km section of watershed is selenium, a known avian and fish teratogen. Native fish were collected via electroshocking from the impacted section of the Mud River and from a control drainage (Left Fork Mud River) during 4 separate sampling events. ICP-MS analysis revealed that the concentration of selenium in skinless fillets from creek chubs (*Semotilus atromaculatus*) was significantly higher ( $6.2 \pm 1.2 \text{ mg Se/kg}$  dry weight,  $p < 0.0001$  by Student's t-test) in the main stem Mud River fish compared to those from the control site ( $2.0 \pm 0.2 \text{ mg Se/kg dw}$ ). Selenium concentrations were  $11.7 \text{ mg/kg dw}$  and  $28.9 \pm 10.4 \text{ mg/kg dw}$  for ovarian and liver tissues respectively while average selenium ovary and liver tissue concentrations were  $4.9 \pm 0.34 \text{ mg/kg dw}$  and  $5.1 \pm 1.5 \text{ mg/kg dw}$  respectively for Left Fork fish. Complementary laboratory studies investigating the trophic transfer of selenium from native Mud River biofilms to fathead minnows (*Pimephales promelas*) are in progress. The zebrafish (*Danio rerio*) is currently utilized as a second laboratory model to investigate mechanisms of selenium mediated teratogenesis. Preliminary results indicate that selenium exposure causes skeletal-muscular deformities at environmentally relevant concentrations and induces glutathione-S-transferase expression.

**WP043 An Empirical Model for Mercury in the South River Aquatic Food Web** C. Mancini, URS Corporation / Manager-Ecosystems Management Team; J.R. Flanders, URS Corporation, URS; R.G. Stahl, DuPont Company / Corporate Remediation Department, DuPont Company / Corporate Remediation Group, DuPont Company / DuPont Corporate Remediation Group. Geomorphological, chemical and biological features of a system all play a role in fate and transport of mercury in the aquatic environment. Methylation is the environmental process that links the abiotic fate and transport of mercury with the aquatic food web. Mercury species [e.g., inorganic mercury (IHg) and methylmercury (MeHg)] differ in their bioavailability and potential toxicity, which further complicates system understanding. Development of accurate food web models for dynamic lotic systems must incorporate the salient features to serve as a useable tool in watershed planning and decision making. A Conceptual System Model (CSM) was developed for the South River, Virginia. The CSM is a somewhat simplified representation of mercury movement in the river from primary sources to high trophic level organisms based on extensive empirical



data. The CSM provides a framework for a numerical quantification of IHg sources and ranking of the pathways (eg., diet vs aquatic) by which MeHg is accumulated in higher trophic level organisms such as fish. The model is also an important tool for integration of disparate data, and stakeholder communication. A key benefit of the South River CSM is an understanding of the link between lower order prey items (eg. invertebrates, and prey fish), and higher order consumers such as fish. The CSM incorporated several different data sets to construct a workable model of the food web, including bioaccumulation models, stable carbon and nitrogen isotope data, fish and invertebrate community data, and site-specific diet studies. In addition, this study used several in situ methods to describe alternate routes of exposure and address uncertainties. The model can be used to identify: Primary mercury sources and pathways to consumers, pathways that may be feasible to intercept to reduce fish mercury levels, uncertainties.

**WP044 Assessing Metal Toxicity Based On a Combined Unit World and Kinetic Modeling Approach** S. Yacoub, University of Toronto / Department of Chemical Engineering and Applied Chemistry, University of Toronto; M.L. Diamond, University of Toronto / Department of Geography and program in planning, University of Toronto / Department of Geography. Ross Lake in Flin Flon, Manitoba, CA has received Zn and Cu enriched mine tailing effluents for over 80 years and is extremely low in biological populations. Computer modeling and field campaigns conducted at Ross Lake have led to the conclusion that current copper concentrations in the effluent are a source of acute toxicity to the lake. In an effort to identify the critical load of metals which may cause toxicity, a Unit World Modelling approach (combining toxicity, speciation and fate calculations) was extended to include kinetic reactions. Discrepancies between initial measured and modeled concentrations highlighted the need for non equilibrium reactions to be included in the model. The intention of the kinetic module is to more accurately estimate the fraction of bioavailable copper and zinc. The purpose of the model is to aid in evaluating toxicity associated with proposed mine closure plan scenarios and mine operations.

**WP045 Bioaccumulation and biotransformation of SeO<sub>3</sub> versus SeO<sub>4</sub> exposed periphyton and subsequent toxicity to the mayfly *Centropilum triangulifer*** J.M. Conley, / Department of Environmental and Molecular Toxicology; D. Funk, Stroud Water Research Center; D. Hesterberg, L. Hsu, North Carolina State University / Department of Soil Science; J. Kim, Stroud Water Research Center; D.B. Buchwalter, North Carolina State University / Department of Environmental and Molecular Toxicology, North Carolina State University / Department of Environmental & Molecular Toxicology. Bioconcentration of selenium oxyanions into primary producers and subsequent biotransformation to organo-selenium forms is considered the predominant driver of food web bioaccumulation and toxic effects in Se-contaminated freshwater ecosystems. Few studies have directly compared selenate (SeO<sub>4</sub>) and selenite (SeO<sub>3</sub>) uptake kinetics into primary producers, and we are unaware of any studies that have done so with complex periphyton biofilms. Further, there is much uncertainty regarding the differential biotransformation of SeO<sub>4</sub> and SeO<sub>3</sub> and subsequent toxicity to consumers. To address these data gaps, we conducted three controlled, time-course, laboratory exposures of natural, complex, freshwater periphyton biofilms to dissolved SeO<sub>3</sub> and SeO<sub>4</sub> under static, static renewal, and static in the presence of elevated total dissolved solids (TDS) conditions. Periphyton uptake of SeO<sub>3</sub> was initially rapid and only slowed when dissolved SeO<sub>3</sub> concentrations decreased. In contrast, periphyton uptake of SeO<sub>4</sub> was initially negligible but began to increase in rate coincident with the occurrence of SeO<sub>3</sub> in the exposure media. We believe this generation of SeO<sub>3</sub> from SeO<sub>4</sub> is the result of microbially mediated dissimilatory reduction as evidenced by the enrichment of Comamonadaceae bacteria based on denaturant gradient gel electrophoresis (DGGE) fingerprinting. Members of this family are known to reduce SeO<sub>4</sub>. Elevated TDS inhibited uptake of SeO<sub>4</sub> but not SeO<sub>3</sub>. X-ray absorption near-edge spectroscopy (XANES) indicated that periphyton Se speciation was identical between the static SeO<sub>3</sub> and SeO<sub>4</sub> exposures after 8d, with the majority (61±6%) of selenium in the (-II) form (i.e., organo-selenides). When fed to *Centropilum triangulifer* mayflies in full-life cycle experiments, there was a similar toxic response with significantly reduced survivorship (SeO<sub>3</sub> exposed periphyton, 73% reduction; SeO<sub>4</sub> exposed, 64% reduction) at the high exposure level (36 µg g<sup>-1</sup>, dw), as compared to controls. Based on the results of our studies, it appears that freshwater periphyton communities absorb dissolved SeO<sub>3</sub> much more readily than SeO<sub>4</sub>. However, microbial reduction of SeO<sub>4</sub> to

SeO<sub>3</sub> appears to be an under-appreciated process that may determine the extent of Se incorporation into food webs where SeO<sub>4</sub> is the predominant species. When bioconcentrated to similar total Se concentrations, SeO<sub>3</sub> and SeO<sub>4</sub> exposed periphyton exhibited nearly identical biotransformation and toxicity to mayflies.

**WP046 Bioaccumulation and Histopathological Alterations in the Bullfrog, *Hoplobatrachus occipitalis* Exposed to Sub Lethal Concentrations of Lead** A. Enuneku, University of Benin / Animal and Environmental Biology; L.I. Ezemonye, University of Benin / Zoology Dept; E.T. Ogbo-mida, National Centre for Energy and Environment / Ecotoxicology and Environmental Forensics. The frog *Hoplobatrachus occipitalis* was exposed to 0.25, 0.50, 1.00 and 2.00mg/l lead for 28 days. There was hepatic bioaccumulation of lead at the end of the exposure period. Bioaccumulation of lead increased significantly (p<0.05) with increase in concentration of the heavy metal. There was high accumulation of lead in the liver which may cause liver damage. At the end of the study, the control frog showed normal structural liver pattern. Frog liver exposed to 0.25 and 0.50mg/l lead after 28 days showed mild congestion of blood vessels. At 1.00 and 2.00mg/l lead exposure, severe congestion of blood vessels was observed which indicates an increase in severity. Severe bile secretion occurred in the 1.00 and 2.00mg/l lead exposures. The observed changes may be due to the direct toxic effects of lead on the hepatocytes. The results of this study showed that *H. occipitalis* manifested hepatic histopathological changes following bioaccumulation after exposure to lead concentrations. There is serious need for the monitoring of lead in aquatic ecosystems. The discharge of effluents containing heavy metals like lead into aquatic ecosystems should be discouraged as this may affect the health of amphibians that are exposed in their habitats. Keywords: Frog, Lead, Liver, bioaccumulation.

**WP047 Bioavailability of Copper Adsorbed to Colloidal Hydrous Ferric Oxide to Freshwater Benthic Grazers** D. Cain, U.S. Geological Survey / Water Resources Division; M. Croteau, C. Fuller, U.S. Geological Survey. Food is a potentially important route of metal uptake for aquatic organisms. This pathway may be especially important for primary consumers (e.g. detritivores and herbivores) because of the high concentrations of metals present in the ingested material (e.g. sediment, seston, and benthic algae). The bioavailability and uptake of metals in these complex food sources will vary with the variety of geochemical phases present in the ingested matter and the digestive physiology of the organism. In this study, the bioavailability of copper (Cu) adsorbed to synthetic colloidal hydrous ferric oxide (HFO) was evaluated. This particle was intended to represent a simple form of inorganic colloid produced during the neutralization of acid mine drainage. HFO has a high capacity to sorb metals and infiltrates benthic substrates such as periphyton where it can be consumed by benthic grazers. The methodology utilized <sup>65</sup>Cu as a tracer to quantify uptake of Cu from HFO by two benthic grazers, a gastropod and a larval mayfly. The labeled colloids were mixed with a food source (the diatom *Nitzschia palea*) to simulate the retention of HFO in periphyton under natural conditions. The uptake of <sup>65</sup>Cu from HFO was determined over a range of environmentally realistic dietary Cu concentrations, and in separate experiments, the bioavailability of Cu was assessed from the calculated assimilation efficiency of the ingested <sup>65</sup>Cu. Desorption of <sup>65</sup>Cu from HFO and mass transfer of <sup>65</sup>Cu to the diatom was examined to support interpretation of the Cu bioavailability from HFO. Results showed that mass transfer of <sup>65</sup>Cu from HFO to the diatom was not appreciable (≤ 3%) under the experimental conditions. Estimates of assimilation efficiency using various methods indicated that the majority of ingested Cu was assimilated (values ranged from 80 to 92 percent for both species). The results indicated that Cu adsorbed to HFO was readily solubilized by digestive processes and subsequently assimilated. This implies that colloidal HFO potentially represents a source of dietary Cu (and possibly other transition metals) to benthic grazers, especially where there is active formation and infiltration of these particles into benthic substrates.

**WP048 Biofilm response to metal contaminated sediment and indirect effects on grazers varies among stream ecosystems** D. Costello, University of Michigan / School of Natural Resources & Environment; A. Burton, University of Michigan / School of Natural Resources & Environment and Cooperative Institute for Limnology & Ecosystem Research. Photo-synthetic biofilms are crucial drivers of many important stream ecosystem functions (e.g., primary and secondary production, N cycling), yet we have a limited understanding of how this critical community responds to



contaminated sediments. We exposed natural biofilm communities in five different streams to a common sediment amended with four concentrations of Ni and Cu. Contaminated sediments in cups were covered with mesh disks and placed directly into streams. Biofilm-colonized disks were analyzed for net primary production (NPP), chlorophyll *a*, metal content, and microelectrode oxygen profiles. Additional biofilm disks were separated from the sediment and fed to *Lymnaea stagnalis* to assess indirect effects of sediment metal on grazers. Among our five streams, we found variation in the biofilm response to metals with the most productive stream showing the strongest response to metal-contaminated sediment. For biofilms that did not show a reduction in NPP, there was still substantial penetration of oxygen into sediments and accumulation of metal in biofilm. *L. stagnalis* exposed to metal-contaminated biofilms fed at a slower rate than those given clean biofilms. This study suggests that biofilms can potentially be impaired by contaminated sediment but the response is context dependent. Further, indirect dietary effects of contaminated sediment occur more widely than direct effects on biofilms. We suggest that further work on biofilm ecotoxicology is crucial to understanding ecosystem-level effects of contaminants.

**WP049 Calculating a Selenium Trophic-Transfer Factor for Chironomus dilutus – A Key Link in Bioaccumulation Estimates** C.J. Rickwood, M. Jatar, Natural Resources Canada / CANMET MMSL; S. Hughes, Shell Health Americas; G. Gilron, Cardero Coal Ltd. Traditional water quality guidelines for Selenium (Se) are not adequate in predicting aquatic toxicity, as the primary route of exposure is dietary. Revision of guidelines to encompass fish tissue-based criteria are currently underway in both the US and Canada. However, a recent analysis of data gaps for translating a fish tissue-based Se criterion to a water-based screening criterion highlighted a number of key research areas that could reduce uncertainty in the calculation. One such area relates to the variability in Se Trophic Transfer Factors (TTF) from periphyton to benthic invertebrates; this is a crucial factor in relating fish tissue guidelines back to water concentrations. This project was initiated to respond to this uncertainty with the primary objective to calculate a TTF for *Chironomus dilutus* when exposed to their Se-spiked plant food source (unicellular, freshwater algae). In addition, known Se-enriched sediment was also incorporated into the experimental design to understand the role of sediments and algae in the accumulation of Se in *C. dilutus*. Test organisms were exposed over a 21-day period, and biological endpoints included survival, growth and Se tissue concentrations. Porewater and overlying water chemistry was measured using “mini-peepers” and tissue chemistry was measured in both *C. dilutus* and their algal food source (*Pseudokirchneriella subcapitata* and *Chlorella kesslerii*). The study results provide an estimate of the relative significance of dietary Se on bioaccumulation in invertebrates and calculate a TTF for this species.

**WP050 Changes in mercury sensitivity by a marine diatom Thalassiosira weissflogii after mercury exposure** Y. WU, W. Wang, Hong Kong University of Science and Technology. Aquatic organisms originating from metal polluted waters always exert differences in sensitivity to metals. However, the underlying physiological or genetic mechanisms of such responses have been less well reported. Therefore, efforts have been made to understand (1) the changes in the Hg(II) or MeHg sensitivity of a marine diatom *Thalassiosira weissflogii* after exposure to different Hg(II) or MeHg concentrations, and (2) the underlying mechanisms. The preconditioned *T. weissflogii* became more tolerant to both Hg(II) and MeHg and the underlying mechanisms may include the changes in cellular mercury accumulation and their detoxification ability. Exposure to higher Hg(II) led to changes in subcellular metal distribution, while exposure to higher MeHg decreased the mercury uptake rate. Other possible physiological effects, including the induction of phytochelatins (PCs) and the changes in cell viability after mercury exposure were also examined. There were significantly different responses of diatoms to Hg(II) and MeHg. These results showed that different strategies were adopted by species to resist different stress.

**WP051 Current levels of trace elements in Pacific cetaceans stranded in Hawaii and examination of metallothionein as a biomarker of trace element exposure** A.M. Hansen, Hawaii Pacific University / College of Natural and Computational Sciences; C.E. Bryan, National Institute of Standards and Technology / Analytical Chemistry Division, Hollings Marine Laboratory; K. West, B.A. Jensen, Hawaii Pacific University / College of Natural and Computational Sciences. Anthropogenic activities can elevate trace elements in marine biota to high levels, potentially harming organisms

and humans as they accumulate in marine foodwebs. Essential trace elements are utilized and regulated by cellular processes, while non-essential trace elements accumulate in tissues and can be highly toxic and persistent. Many cetaceans feed at high trophic levels similar to that of humans, will bioaccumulate and biomagnify certain contaminants, and are often considered sentinels for human health. Levels of trace elements can vary with age, foraging range, body condition, and diet preference. Exposure to high levels of trace elements is considered a contributing factor in marine mammal population declines, increased incidences of disease, and unusual mortality events. Hawaii is an increasingly important geographic location for global monitoring, yet trace element levels in Hawaiian cetaceans have not been reported to date. This study measures essential and non-essential trace elements As, Cd, Cu, Cr, Hg, Mn, Pb, Se, Sn, Sr, and Zn in hepatic tissue of 15 species of stranded Hawaiian cetaceans (n=32), collected and banked by the Hawaii Pacific University Marine Mammal Stranding Program from 1997-2011. Measurements of trace elements were carried out using high resolution inductively coupled plasma mass spectroscopy (HR-ICP-MS), and atomic absorption spectrophotometry (AAS). Trace element concentration results in striped dolphins (*Stenella coeruleoalba*, n=7) were found to be within concentration ranges for this species in other ocean regions. Hg and Se concentrations ranges (0.53-261 µg/g, w.w. and 2.0-79.3 µg/g, w.w., respectively) are similar to concentration ranges found on the coast of Japan. The highest levels of Hg, Se, Cd, Sn, and Pb were found in an adult female false killer whale (*Pseudorca crassidens*), at levels that suggest possible health effects. Metallothionein, a protein biomarker associated with Cd, Cu, Hg and Zn, was measured by western blotting. The results of this study establish initial trace element concentration ranges for many of the cetacean species residing in the Central North Pacific, provide insight into the biology of Hawaiian marine mammal populations, and contribute to our understanding of anthropogenic impacts in Hawaiian ecosystems.

**WP052 Developing a site specific understanding of the toxicity of rare earth elements to Daphnia pulex and Hyalella azteca** O. Vukov, / Department of Biology, Wilfrid Laurier University / Biology; J. McGeer, Wilfrid Laurier University / Department of Biology. Lanthanides or rare earth elements (REEs) occur as either the light or heavy REEs. Contrary to their name, REEs are abundant in the earth crust although concentrations show a general trend of decrease with increasing atomic number. The toxicological understanding of REEs in the aquatic environment is very limited. The objective of this research is to compare the toxicological effect of REEs to the freshwater invertebrates *Daphnia pulex* and *Hyalella azteca* and to understand the toxicity modifying influence of Ca, Mg, Na, and dissolved organic carbon (DOC). In this study cerium (light REE) and dysprosium (heavy REE) were chosen as a result of preliminary studies and a biotic ligand modeling approach was applied to link speciation with toxicity. Standard methods (Environment Canada) were used for testing and culture; pH 7.0, Ca 0.18, Mg 0.14 (mM) and 21 °C for *D. pulex*, and pH 7.2, Ca 0.1, Mg 0.03 (mM) and 21 °C for *Hyalella*. Acute toxicity tests, with *D. pulex* and with 2-9 d old offspring in 240 ml of solution for 96 h for *Hyalella*. Atomic absorption certified stock solutions was used to create exposure solutions which were prepared in duplicate and pH adjusted (when necessary) 24 h prior to test initiation. On a nominal basis the EC50s were 2540 (95% confidence interval 2170-2960) µg Ce/L for *Daphnia* and 72.1 (54.4-95.4) µg Ce/L for *Hyalella*. In a series of tests the protective effect of cationic competition was tested with Ca (0.1 to 2.0 mM), Mg (0.03 to 0.5 mM) and Na (0.1 to 2.0 mM). As well, the ability of natural organic matter to complex Ce and reduce bioavailability was tested at DOC concentrations of 4 and 10 mg C/L. Ca provided significant protection against Ce toxicity, the 96 h EC50 for *Hyalella* increased from 72.1 µg Ce/L to 623 (476-814) µg Ce/L when Ca in the test solution was increased from 0.1 mM to 1.0 mM. An understanding of the effects of water chemistry on the toxicity of REEs to sensitive freshwater invertebrates will contribute towards the development of site specific approaches for setting water quality criteria/guidelines and discharge objectives. This research is supported by Environment Canada.

**WP053 Ecologically Framed Mercury Database of Finfish from Lower Chesapeake Bay** X. Xu, College of William and Mary, Virginia Institute of Marine Science / Environmental and Aquatic Animal Health.; M.C. Newman, College of William and Mary, Virginia Institute of Marine Science / Environmental and Aquatic Animal Health; M.C. Fabrizio, College of William and Mary, Virginia Institute of Marine Science / Fisheries Science. Mercury in fish and shellfish is a major exposure pathway for humans.

To generate accurate fish consumption advisories and make appropriate risk judgments, it is crucial to understand determinants of mercury accumulation in fish such as trophic ecology, fish age, and migratory behavior. The ability to interpret data from a trophic vantage is helpful to understanding and predicting variations in mercury concentrations among finfish species, locations, and years. Between 2009 and 2011, we sampled 348 individuals representing nine finfish species (striped bass, white perch, Atlantic croaker, weakfish, spot, American eel, summer flounder, white catfish, and blue catfish) from the lower Chesapeake Bay, and the James, York, and Rappahannock Rivers. Total length was measured from each fish and a section of axial muscle was removed for total and methyl mercury analysis. We measured stable isotopes ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) in the muscle plug to understand trophic position; migratory behavior was gauged with otolith Sr:Ca molar ratios. (1) We found no evidence of potential human harm from mercury consumption: no sampled species had total mercury concentrations approaching the human health screening value (300 mg/kg wet weight). (2) A methyl mercury biomagnification model was constructed ( $\text{Log}_{10}\text{MeHg}=0.47\delta^{15}\text{N}-5.48$ ), where the slope (0.47; 95%CI: 0.27-0.67) indicated the influence of trophic position on mercury biomagnification. (3) Methyl mercury increased with increasing  $\delta^{15}\text{N}$ , but there was no obvious relationship between methyl mercury and  $\delta^{13}\text{C}$ . Especially inconsistent with the overall observed pattern was Atlantic croaker, which exhibited low values of  $\delta^{15}\text{N}$  and mercury concentrations above those predicted by the model. This species may be exposed to different mercury sources as juvenile stages migrate from brackish waters into more saline environments (4) Atlantic croaker that inhabited less saline environments (Sr:Ca molar ratio < 4) tended to accumulate more mercury than those exposed to more saline waters (Sr:Ca molar ratio > 8.5-9). There was no Sr:Ca related trend in mercury concentration for striped bass of the size range sampled.

**WP054 Effects of (binary) mixtures and soils containing heavy metals on the activity of seed germination and bacterial bioluminescence and gene mutation** I. Kong, Yeungnam University / Environmental Engineering; K. Ko, KIGAM. The purpose of this investigation was to assess the joint effect of metal binary mixtures, as well as the toxic effect by metal contaminated soils, to the activity of the seed germination, bacterial bioluminescence and gene mutation. To investigate the joint metal effect, one toxic unit (1 TU) approach was performed to predict which metal will contribute to adverse effects on each bioassay. Different sensitivities and orders of toxicity were observed among bioassays on mixture metals tested. In general, the toxicity of binary mixture with arsenite, which was highly toxic as single, was greater than others. According to 1 TU approach with the equal mixture, antagonistic additive effect was mostly observed in this investigation. Overall, the combined effects of heavy metals among different bioassays are hardly generalized since they depend on both the kind of chemical and the organisms used in each bioassay. However, the results indicated that a battery of bioassays and the mixture effects rather than one bioassay and single effects may comprise a better tool for the bioassessment of environmental pollutants.

**WP055 Evaluation of aluminum chronic toxicity to the fathead minnow and zebrafish using a flow-through pH-control toxicity test system** A.S. Cardwell, Oregon State University / Faculty Research Assistant; B.A. Stubblefield, Oregon State University, Oregon State University / Environmental and Molecular Toxicology; B.J. Adams, Rio Tinto / Product Stewardship; B.W. Gensemer, GEI Consultants / Ecological Division; E. Nordheim, EAA; R.C. Santore, HDR|HydroQual, Inc. The toxicity of some metals, such as aluminum, is a function of their speciation and this is a function of predominately environmental pH. To evaluate the acute and chronic toxicity of metals, whose speciation is a function of water pH, it is necessary to continuously monitor and control water pH throughout the conduct of a toxicity test. Previous chronic toxicity tests with aluminum were typically conducted under acidic test conditions given that the research was conducted to address concerns associated with acid rain. Few studies have been conducted at pHs more typical of natural surface waters. To address this data gap, fathead minnow and zebrafish flow-through early-life stage toxicity tests were conducted using an aged stock solution of aluminum nitrate nonahydrate ( $\text{Al}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ ) for 33- and 35-days, respectively. Hydrogen-ion concentrations were maintained through the use of a pH-control system with proportional output. The control/dilution water was well water blended with reverse osmosis water to achieve a nominal hardness of 100 mg/L as  $\text{CaCO}_3$ . The control water, as well as the aged aluminum

stock solution, was pH controlled by direct injection of dilute HCl to achieve a constant pH of 6.0. During the conduct of the tests, pH was maintained within 0.2 units prior to entering the test chamber and within 0.4 units within the test chamber. A 10% growth effect (EC10) on the fathead minnow and zebrafish was determined to be 417.4 and 150.1  $\mu\text{g/L}$  total aluminum, respectively.

**WP056 Evaluation of heavy metals in salt leaves from glasswort (*Salicornia bigelovii*) in lower Galveston Bay, Texas** C.L. Howard, University of Houston-Clear Lake / Department of Biological & Environmental Sciences. Tidal wetlands are among the most productive ecosystems in North America. In Gulf of Mexico estuaries, the perennial glasswort, *Salicornia bigelovii*, is common in salt flats associated with these wetlands. As an adaptation to the high salinities characteristic of salt flat sediments, *Salicornia* shunts salts to specific leaves on the plants, which then drop off, reducing the salt concentration in the plant. Salt excretion as a mechanism for reducing heavy metal burdens has been investigated in other halophytes, but not in glasswort, although *Salicornia* populations are found in areas variously contaminated with a number of different heavy metals. The objective of this study was to identify and quantify six heavy metals (Pb, Cd, As, Cu, Zn, and Sn) in *Salicornia* collected from 10 sites along the lower Galveston Bay system varying in the type, extent and history of heavy metal contamination. Twelve plants and three sediment samples were collected from each site. Field parameters, growth metrics, and chlorophyll (SPAD-1500) data were recorded on site. Leaves, shoots, roots and sediments were acid digested and metal concentrations were measured by ICP. Plants from different sites exhibited heavy metal patterns similar to the sediments in which they were growing. The salt leaves from all plants contained significantly higher concentrations of metals than did the other plant tissues or the sediments, indicating that *Salicornia* can concentrate and remove heavy metals via their salt leaves.

**WP057 Macrophage Centers: A Synthesis of Their Implications for Mercury Fate and Toxicity in Fish** A. Roberts, University of North Texas; M.M. Chumchal, Texas Christian University / Biology department; P. Drevnick, Université du Québec / INRS-ETE; K. Kidd, Canadian Rivers Institute, University of New Brunswick / Biology, University of New Brunswick / Biology department; B. Moore, Louisiana Tech; D.H. Adams, Florida Fish and Wildlife Conservation Commission / Fish and Wildlife Research Institute, Cape Canaveral Scientific, Inc. / Fish & Wildlife Research Institute, Florida Fish & Wildlife Commission / Fish & Wildlife Research Institute; B. Barst, Université du Québec / INRS-ETE; J. Smith, University of North Texas, University of North Texas / Senior Scientist/Project Manager; K. Batchelar, University of New Brunswick. Melanomacrophages, fixed tissue macrophages of amphibians and fish, have been used as biomarkers of general pathology but also occur as non-pathologic germinal centers of fixed macrophages. Melanomacrophage centers (MMCs) follow an evolutionary pattern in distribution and degree of organization with a trend from random distribution and low levels of organization in Chondrichthyes and Agantha, to highly organized structures in the liver of the primitive bony fish, and finally to well-organized structures in the spleens and kidney of the teleosts. Increased MMC occurrence has been reported to correlate with mercury exposure in fish but previous work has done little to assess the role of MMCs in potential metabolism or toxicity pathways. Due to the widespread nature of mercury contamination and its movement through food webs, phylogenetic and species-specific differences in mercury toxicity/metabolism have important consequences for potential impacts on both humans and wildlife. The goal of this presentation is to provide a synthesis of several studies conducted examining MMCs in livers of fishes ranging from primitive to more recently diverged species across a wide geographic area (including Canada, the Great Lakes, and several sites in the southern United States). Many of these studies examined histology, total mercury content, mercury speciation, cellular mercury-sequestration by laser ablation ICP-MS, as well as other endpoints. We found that, generally, MMC size/occurrence was highly correlated with total liver Hg and MMCs accumulate total Hg at 2-4 times the concentration of adjacent hepatocytes. We also found that some primitive bony fish sequestered mercury in the liver at concentrations in excess of 30,000 ppb w.w. These fish also had a high percentage of inorganic mercury in the liver relative to methyl mercury. Taken together, these studies indicate that MMCs are an important component of the toxicology of mercury in bony fishes and illustrate the need for focused studies on liver function, immunocompetency, and reproduction.



**WP058 Relationships Between Selenium Concentrations in the Insect *Chaoborus* and Selenium Speciation in Lakewater** D.E. Ponton, Institut National de la Recherche Scientifique / Eau, Terre et Environnement, Institut National de la Recherche Scientifique / ETE, INRS-ETE / Eau, Terre et Environnement; L. Hare, INRS-ETE. To better understand the influence of Se speciation on Se bioaccumulation, we measured Se speciation in the waters of 16 eastern Canadian lakes and compared it to Se concentrations in a potential biomonitor. The relative importance of selenite (Se(IV)), selenate (Se(VI)), and organo-Se varied with lakewater pH, that is, percentages of selenate and organo-Se were higher in acidic lakes and those of selenite were greater in alkaline waters. Using a chemical speciation model, we show that the concentrations of two forms of selenite ( $\text{HSeO}_3^-$  and  $\text{SeO}_3^{2-}$ ) change with lakewater pH such that at pHs >6, the proportions of  $\text{HSeO}_3^-$  and organo-Se decrease whereas those of the  $\text{SeO}_3^{2-}$  and selenite increase. We estimated the potential bioavailability of the various Se species by comparing their concentrations in lakewater to those in larvae of the phantom midge *Chaoborus*. Selenium concentrations in this insect were significantly correlated with those of total dissolved Se as well as with those of selenite, selenate and organo-Se. We obtained an even stronger correlation by adding the concentrations of organo-Se to those of selenate and correcting for hypothesized competition between selenate and sulfate at biological uptake sites. We conclude that larvae of *Chaoborus* would be an effective biomonitor for Se in lakewater and in planktonic food webs.

**WP059 Study of the efficiency of coal in the adsorption and reduction of its copper ecotoxicity** L. Morais, Sao Paulo State University – UNESP / Environmental Engineering; R. Fracacio, Sao Paulo State University – UNESP / Environmental Engineer; A. Toledo, Padre Anchieta University/ São Paulo University/Nuclear and Energy Research Institute / Chemical Engineering; F.G. Junior, Padre Anchieta University; A.H. Rosa, Unesp / Environmental Engineering; C. Watanabe, P. Tonello, L. Farceto, V. Campos, Unesp. The aim of this study was to evaluate the adsorption capacity of copper metal by activated carbon from orange peel, dried (110°C) and pyrolysed at different temperatures (250°C, 350°C and 450°C) and imply if these adsorptions in reducing toxicity for acute and chronic test organisms *Ceriodaphnia dubia* (microcrustaceans) and *Pseudokirchneriella subcapitata* (seaweed). The test solution was prepared starting from copper sulfate (99% purity) and water reconstituted from distilled water (hardness 44mgCaCO<sub>3</sub>L<sup>-1</sup> and pH 7.4). The initial concentration of copper was 1.18 mg.L<sup>-1</sup>, detected in atomic absorption spectrometry (AAS). Experiments to evaluate the adsorption of copper to the different coal was performed with two replicates at 1 liter containers. In each replica maintained at ratio of 0.5g of charcoal/liter of test solution of copper. These solutions remained under stirring for 24h and then was vacuum filtered on a Millipore membrane (GF/C) de 0.45µm mesh. Then an aliquot of 5 ml was taken for reading the concentration of the metals as described above, and the remainder was used in toxicity tests with *C. dubia*, during eight days, to evaluate the survival and the number of offspring per individual, and *P. subcapitata* exposed for 72h evaluating the number of cells compared to control. The results demonstrated that the adsorption orange peel presented with high adsorption capacity of the metal copper ranging from 61% (dry coal) and 96% (pyrolysis at 250°C). Among the coals pyrolyzed, the lowest absorption capacity obtained was 90% at 450°C. However, among the coals pyrolyzed, ecotoxicological no difference between treatments, with no acute and chronic toxicity, considering both species. After treatment with dry charcoal, there were significant decrease in the number of neonates *C. dubia*, indicating that although the efficiency of chemical reduction, do not satisfactorily presented to reduce toxicity.

**WP060 The DOM of *Alnus glutinosa* and *Acer negundo* leaves. Influence on the toxic effects of copper to the *L. minor*.** R. Karitonas, Institute of Botany / Institute of Botany, Institute of Botany / Laboratory of Hydrobotany; S. Jurkonienė, Nature Research Centre / Institute of Botany; A. Karitonaitė, J. Kostkevičienė, Vilnius University; L. Manusdzianas, Nature Research Centre / Institute of Botany; K. Sadauskas, Lithuanian University of Education. Bioavailability and toxicity of some metals in natural surface waters are reduced by complexation with DOM. The purpose of this study was to examine how the presence of DOM from *Alnus glutinosa* and *Acer negundo* leaves, which exhibited a different tannin and total polyphenol contents, may affect Cu toxicity *L. minor*. Effect of DOM from *A. glutinosa* and *A. negundo* leaves on Cu toxicity was examined in *L. minor* under laboratory conditions. The growth reaction of one was studied at control and 4 samples

(1- no added DOM +Cu(100µM); 2- DOM (100 mgC/l) + no added metal; 3- DOM(100 mgC/l)+Cu(100 µM); 4- DOM(10 mgC/l)+Cu(100 µM)). The fronds area and root length was assessed after 7 d exposure. The level of lipid peroxidation in plant tissues was determined as MDA – after treatment for 0,75, 1,5, 3, 6, 12, 24 and 48 h. The results indicate, that compared to the control, *L. minor* root length significantly affected by all treatments. Control –2338mm, 1 – 0mm, *A. glutinosa*; 2 – 420mm, 3 – 14mm, 4 – 36mm; *A. negundo*: 2 – 828mm, 3– 417mm, 4– 97mm. At Cu 100 µM concentration, *L. minor* fronds area was reduced near 97%. Whereas in *A. glutinosa* and *A. negundo* 3 and 4 treatments, fronds area growth was reduced in the range of 64–79%, when *L. minor* were exposed only to DOM from *A. negundo* leaves, a slight decrease of fronds area was 2%. Presence of DOM from *A. glutinosa* leaves resulted in a significant decrease of fronds area (41 %). After 6 h, the curve of MDA content in *Lemna* tissues with *A. negundo* 3 and 4 treatments was similar to that of Cu treatment. MDA content increased gradually with the increased time of exposure. While responses of MDA activity exposed to *A. glutinosa* 3 and 4 treatments was reduced in the range 15–50% to that exposed to Cu. The response of antioxidant enzymes, such as CAT, APX, guaiacol peroxidase and GR were also examined. After 24 h and 48 h, only guaiacol peroxidase activities significantly recovered to control levels in all treatments. This work successfully demonstrated that DOM from *A. glutinosa* leaves decreased Cu bioavailability and toxicity to *Lemna* for 48 h. In contrast, DOM from *A. negundo* leaves did not show any apparent effect for lipid peroxidation in plant tissues. DOM (100 mgC/l) from *A. glutinosa* leaves inhibits *L. minor* root length and fronds area growth, DOM from *A. negundo* leaves inhibits only one root length.

**WP061 The role of aquaporin 3 in the uptake of arsenite through the intestine of the Atlantic killifish (*Fundulus heteroclitus*)** D. Jung, Dartmouth Medical School / Physiology, Geisel School of Medicine at Dartmouth / Microbiology and Immunology, Physiology, Dartmouth Medical School / Microbiology and Immunology; M.A. Adamo, R.M. Lehman, Mt. Desert Island Biological Laboratory; R. Barnaby, Geisel School of Medicine at Dartmouth; B.P. Jackson, Dartmouth College; J.R. Shaw, Indiana University / The School of Public and Environmental Affairs; B.A. Stanton, Geisel School of Medicine at Dartmouth / Microbiology and Immunology, Physiology. Aquaglyceroporins (AQPs) are proteins that mediate movement of water and small solutes across cellular membranes. Previously, we cloned kAQP3a from the gill of the Atlantic killifish (*Fundulus heteroclitus*), an environmental sentinel species. kAQP3a, the only AQP expressed in gill, is the first AQP3 described that does not transport arsenite. This finding accounts for the low levels of cellular arsenite in gill of killifish exposed to environmental arsenite. In addition, a second homolog of AQP3 was identified as the consensus from a transcriptome database, and this AQP3, kAQP3b, transports arsenite. In this study, we performed a set of experiments to identify the AQP3s in the intestine, a major route of arsenite uptake, that mediate arsenite assimilation. First, we examined AQP mRNA expression by quantitative RT-PCR in the killifish intestine. Among the aquaglyceroporins examined (AQP3, AQP7 and AQP9), only kAQP3 was significantly expressed above background levels. Western blot studies with a polyclonal antibody that did not discriminate among kAQP3 variants, revealed that kAQP3 abundance was higher in killifish acclimated to FW compared to SW. Intriguingly, whereas only kAQP3a was expressed in the intestine of FW killifish, both kAQP3a and a new variant, kAQP3c, were expressed in the intestine of SW fish. Moreover, when this new variant was transfected into HEK293T cells, cells took up arsenic as effectively as cells transfected with kAQP3b. To test the hypothesis that increased expression of this variant in SW fish compared to FW fish will increase arsenic levels in the killifish intestine, we examined arsenic levels in the intestine of FW fish and SW fish exposed to 1000 µg/L arsenite for 72 hrs. The amount of arsenic detected in the intestine of SW fish was higher than the amount detected in FW fish. Together, our results indicate that arsenite uptake in the killifish mostly likely occurs via ingestion, and that killifish acclimated to SW take up more arsenite than FW acclimated fish, even though total AQP3 protein abundance is reduced, because kAQP3c expression is up regulated in SW fish and because SW fish drink more water than FW fish.

**WP062 Understanding the metal resistance in oysters *Crassostrea hongkongensis*: the importance of metal biokinetics** K. PAN, W. WANG, The Hong Kong University of Science and Technology. Oysters *Crassostrea hongkongensis*, a widely cultivated oyster species in Southern China, demonstrate



extremely high capacity for accumulating metals. The metal concentration in contaminated *C. hongkongensis* (blue oysters) can reach a level up to 3% of body dry weight, especially for Cu and Zn. It remains unknown that how they build up such high metal concentrations in their bodies and how they develop metal resistance in contaminated environment. A seven month transplantation experiment was conducted to re-construct the process how the oysters counteract metal stress during the early stage of metal contamination. The metal concentrations increased substantially in the oysters transplanted from a pristine site to a contaminated site. The metal biokinetics in the oysters dramatically changed after suffering from metal stress. The clearance rate, dissolved uptake rate (for Cd and Zn), and metal assimilation efficiency (for Zn) was depressed, while the metal efflux rate (for Zn) was enhanced in the contaminated oysters. Beside the change of metal homeostasis, the oysters were able to sequester metals into subcellular non-toxic forms and maintain a low portion of metals distributing in the metal-sensitive fraction. This comparative bioaccumulation study on *C. hongkongensis* suggested that adjustment of metal biokinetics played an important role in the survival of oysters in metal contaminated environment.

**WP063 Use of Sulfur Content as a Surrogate Measure of Mercury binding capacity in fish Tissues** J. Li, K.G. Drouillard, University of Windsor / Great Lakes Institute for Environmental Research; D. Haffner, University of Windsor / GLIER. Organic contaminants accumulate primarily in the lipids of biological tissues and therefore concentrations of organic contaminants, such as PCBs, are often expressed on a lipid normalized basis. This permits evaluation of chemical potentials across samples that vary in partition capacity and provides more meaningful interpretation of concentration differences across tissue samples. Like PCBs and other hydrophobic organic chemicals, mercury possesses the ability to biomagnify in food webs. Methylmercury (MeHg), accounts for more than 90% of Hg in fish and was originally thought to bind to lipids similar to hydrophobic contaminants. However, recent studies have shown that Hg in fish is primarily bound covalently to sulfhydryl groups in cysteine rich proteins. Yet mercury concentrations are typically expressed either on a wet sample weight or dry sample weight basis and exhibit high variability when compared against different tissue samples. In this study, it is hypothesized that total sulfur content in biological tissues can be used to provide a surrogate measure of both the cysteine content and binding capacity of tissues for methylmercury. Total Hg concentrations were analyzed in gonad, liver, dorsal muscle and carcass of walleye (*Sander vitreus*), white Bass (*Morone chrysops*), longnose gar (*Lepisosteus osseus*), channel catfish (*Ictalurus punctatus*) and golden redhorse (*Moxostoma erythrurum*) collected from Detroit River, summer 2011. Under the condition that mercury concentrations are in equilibrium between different tissues within the same individual, it is predicted that sulfur normalized Hg concentrations will be similar across different tissues of the same organism and exhibit lower inter-tissue variability compared to dry-weight and lipid-corrected mercury concentrations. The ratio of gonad/carcass, liver/carcass and dorsal muscle/carcass was 0.3636( $\pm 0.1617$ ), 5.5198( $\pm 10.3994$ ), 2.3972( $\pm 1.5841$ ) for wet weight concentration, and 0.4591( $\pm 0.2051$ ), 7.5202( $\pm 15.3918$ ), 2.9264( $\pm 1.9286$ ) for lean dry weight concentration, respectively. Sulfur content and cysteine analysis is projected in the near future. We predict that sulfur content normalized tissue/carcass concentration will approach 1:1 ratio making sulfur content a good surrogate measure of Hg partitioning in fish.

**WP064 Variation in biochemical composition of *Chlorella vulgaris* grown under different combinations of phosphate and copper** I. de Souza, Federal University of Sao Carlos (UFSCar); A.T. Lombardi, UFSCar / Botany; M.G. Melao, UFSCar / Hydrobiology. Microalgae physiology responds to the environmental conditions. In this research we investigated the effect of combinations of phosphorus (P) and copper (Cu) on the growth, biochemical composition and Cu toxicity to the freshwater microalgae *Chlorella vulgaris* under controlled laboratory conditions. Copper was analyzed in free ionic form that is the bioavailable form. *C. vulgaris* cultures were acclimated to each treatment's specific P concentration to be tested in combination with Cu. We considered cells were acclimated to a specific P concentration after its growth rate had been stabilized for at least four generations, always transferring the cells while in the beginning of the exponential growth phase. Biomass and physiological parameters analyzed were cell number (cell.mL<sup>-1</sup>), chlorophyll-a concentration, dry weight, lipid classes (Iatroscan TLC/FID), and total cellular proteins and carbohydrates at the combinations of P/Cu. Phosphate concentrations tested ranged from

5.0x10<sup>-5</sup> to 1.0x10<sup>-6</sup> mol.L<sup>-1</sup> and free Cu<sup>2+</sup> ions concentrations ranged with in 1x10<sup>-10</sup> and 5x10<sup>-8</sup> mol.L<sup>-1</sup>, and were determined through ion selective electrode (ISE). Our results showed that Cu toxicity to *C. vulgaris* increased at low P. Carbohydrate, protein and lipid productions were in general triggered at low P and high Cu.

**WP065 Is Reduction of Selenium Contamination in Coal Mining Produced End-Pit Lakes Possible through Ecosystem Manipulation?** A. Luek, University of Lethbridge; C. Brock, Alberta Environment; D.J. Rowan, Atomic Energy of Canada Limited; J.B. Rasmussen, University of Lethbridge. Selenium (Se) is a trace nutrient, which, at elevated concentrations, leads to severe impacts on aquatic ecosystems, including teratogenic deformities and death in fish. Of particular concern are end-pit lakes, a result of abandoned, open-pit coal mines filling with water, which collect waste-rock leachate high in Se and act as a Se source to downstream environments. Although management policies limiting the discharge of Se exist, efficient large-scale remediation methods are scarce and often cumbersome. We tested a whole-lake method of reducing Se concentrations in the waters of end-pit lakes by manipulating the anaerobic bacterial community present in the lakes' sediments with fertilization of the water column. This bacterial community can preferentially substitute Se for sulfur (S) during anaerobic respiration and reduce selenates and selenites to elemental Se, thereby providing a potential method of trapping Se in lake sediments. To enhance the anoxic habitat favorable for the bacteria, the end-pit lakes were fertilized to a highly eutrophic phosphorus concentration of ~0.75-1 mg/L. Fertilization drastically increased primary production in the epilimnion, while respiration of the resultant phytoplankton bloom, combined with their shading effects, created anoxic conditions below the euphotic zone. Increased amounts of organic matter, which sank into the anoxic zone, served as a carbon source for the bacterial community. The resultant increase in bacterial growth and abundance ultimately caused a reduction in the amount of Se in the water column due to anaerobic respiration of Se favored over S. This experiment followed a pulse manipulation approach where the Se reduction process is considered a passive treatment methodology. Successfully applied, this method is suitable for enclosed end-pit lakes with long water retention times and deep basins. It will not only reduce Se contamination in afflicted lakes but potentially initiate the overall productivity of the aquatic ecosystem, making it suitable for further reclamation steps, such as fish stocking and recreational uses.

**WP066 Linking subcellular metal partitioning to the particulate and dissolved exposure pathways and chronic effect of copper in two deposit feeder organisms** O. Campana, Institute of Marine Science Andalusia; S. Simpson, CSIRO Land and Water; A. Taylor, University of Canberra; J. Blasco, Institute of Marine Science Andalusia; W.A. Maher, University of Canberra / Dept. of Health, Design & Science. Metal toxicity occurs when organisms cannot cope with an overwhelming influx and the subsequent accumulation rates of metals. To predict the effects of metals it is necessary to understand the factors that influence the rate of uptake from the various exposure routes. Particularly in the case of chronic toxicity, it is useful to also understand the sub-cellular partitioning of metals. Following uptake, metals may partition within different sub-cellular tissues, be detoxified or excreted, but only the metabolically active fraction of metal contributes to toxicity. Toxicity is elicited when the metabolically active fraction reaches a critical level in one or more sensitive compartments of the organism. The present study investigated the links between the sub-cellular metal exposure and toxic effect to reproduction of the epibenthic amphipod *Melita plumulosa* and growth of the bivalve *Tellina deltoidealis* exposed to copper-contaminated sediments with varying properties (particulate organic carbon (POC) content and/or fraction of fine particles). The sub-cellular partitioning of copper was determined for both organisms and residue-effect relationships investigated. Strong correlations existed between (i) total copper bioaccumulation and both POC-normalized copper concentration of the *T. deltoidealis* and both the %-POC/silt normalized sediment copper concentration and copper concentration in the water phase; and (iii) the sublethal effect and metabolically available fraction for *M. plumulosa*. Copper associated with the particulate phase has been demonstrated to be the major exposure route and potential cause of the toxicity for *M. plumulosa*. However, in *T. deltoidealis*, particulate and dissolved exposure routes seem to play both an important role in copper uptake. These sub-cellular copper partitioning results provide further insight into the interaction between tissue residues and chronic effects.

**WP067 Mercury accumulation in cartilaginous fish from Rhode Island's coastal waters** N.J. Kutil, D. Taylor, Roger Williams University / Marine Biology. Mercury (Hg) is a toxic environmental contaminant that bioaccumulates in fish tissues, including numerous marine species. Cartilaginous fish of the subclass Elasmobranchii are important ecological constituents of marine ecosystems, yet the fate of Hg contaminants in their body tissues is largely unknown. In this study, four species of elasmobranchs: little skate (*Leucoraja erinacea*), winter skate (*L. ocellata*), smooth dogfish (*Mustelus canis*), and spiny dogfish (*Squalus acanthias*), were collected from Rhode Island/Block Island Sound, and the Hg content (ppm wet wt) of white muscle tissue was analyzed using automated combustion atomic absorption spectrometry. Diet and feeding habits for each species were also assessed by stomach content and stable nitrogen ( $\delta^{15}\text{N}$ ) and carbon ( $\delta^{13}\text{C}$ ) isotope analyses. Mean Hg concentrations differed significantly among species, with highest levels measured in smooth dogfish, followed by spiny dogfish, and little and winter skates respectively. The Hg concentration of skate muscle tissue did not vary by body weight, suggesting that Hg does not bioaccumulate in these species. Conversely, smooth and spiny dogfish both bioaccumulate Hg with respect to body size, although smooth dogfish have a higher Hg content relative to spiny dogfish. The elevated Hg concentration of smooth dogfish may be explained by their higher trophic level status, as determined from  $\delta^{15}\text{N}$  signatures. The enriched  $\delta^{13}\text{C}$  values of skates and smooth dogfish indicated benthic foraging, which was further confirmed by the dominance of decapods and crustaceans in the stomach contents. Conversely, squid and butterflyfish were the principal prey of spiny dogfish, and the contribution of these pelagic prey was reflected in the depleted  $\delta^{13}\text{C}$  signature. Future work includes researching the effect of habitat use, analyzing prey Hg, and analyzing Hg concentrations of the liver to better understand bioaccumulation patterns in these species.

**WP068 The Effect of Water Hardness and Composition on the Toxicity of Sulphate to Fish (*Oncorhynchus Mykiss*) and Invertebrates (*Daphnia Magna*)** C.J. Kennedy, Simon Fraser University / Department of Biological Sciences; C. Meays, G. Butcher, K. Rieberger, BC Ministry of Environment; C.J. Schwarz, Simon Fraser University. Studies have suggested that water hardness and its composition play a pivotal role in modifying the toxicity of a number of environmental contaminants. The lethal and sublethal effects of sulphate ( $\text{SO}_4^{2-}$ ) under varying water hardness values and composition (Ca:Mg ratio) were examined in *Daphnia magna* and rainbow trout. The acute toxicity of  $\text{SO}_4^{2-}$  to *D. magna* was examined in 48-h EC50 tests and in 96-h LC50 tests with rainbow trout eggs and fry. Chronic toxicity tests with *D. magna* were 21-d survival and reproduction tests, and with trout were 45-d survival and growth tests. For each test, a range of 5 water hardness values were used with three compositions: 100:0, 50:50, and 0:100 [Ca:Mg]. Model averaging was used to determine the relative support for various hypotheses regarding the effect of hardness and composition on the concentration ( $\text{SO}_4^{2-}$ )-response curve. Model average estimates of benchmark concentrations (e.g. EC50) were also obtained. Data from the fish and invertebrate mortality studies were fit by relatively simple probit models, showing that the concentration-response curves for the different hardness levels were well separated, with decreasing  $\text{SO}_4^{2-}$  toxicity at higher water hardness values. It appears that the protective effects of hardness plateau after a certain hardness level. There was some evidence of hardness composition effects, but the effects of composition were small relative to the effects of the different hardness levels. Modeling the results from the chronic studies were not as clear; the data often showed no simple shape for the responses, and different hardness and composition did not show a consistent pattern. A three-parameter logistic model provided an acceptable fit for the specific growth rate of fish, number of broods in, and number of offspring in *D. magna*. The effect of composition was much smaller than the protective effect of hardness on the specific growth rate of fry, number of offspring and number of broods. This study indicates that water hardness has a protective effect against both the acute and chronic toxicity of  $\text{SO}_4^{2-}$  in fish and invertebrates, with lesser modifications due to composition. It also highlights the importance of choosing appropriate endpoints for assessment, as these modifying effects were different between acute and chronic tests.

**WP069 Method Refinements for the 10-d Midge Test: Defining a Modified Feeding Regime for 10-D Exposures with *Chironomus dilutus*** T.J. Norberg-King, U.S. EPA / ORD, NHEERL, Mid-Continent Ecology Division, US EPA / Mid-Continent Ecology Division; T.L. Highland, US EPA / ORD, NHEERL, Mid-Continent Ecology Division; J.R. Hockett,

US EPA; D.R. Mount, US Environmental Protection Agency / ORD; T.W. Valenti, Syngenta Crop Protection LLC / National Research Council, National Research Council / U.S. Environmental Protection Agency. From a compilation of historical control performance data from 10-d tests with midge (*Chironomus dilutus*) conducted in our laboratory, we observed a tendency towards increased growth in replicates with fewer individuals present. This pattern suggests that food limitation may influence growth in this test, which could be a particular concern if evaluating sediments with differing innate nutritional content. To better understand and address this issue, we conducted a series of experiments with the larval midge using different organism densities, diets, and substrates. An initial study in which stocking density was varied from 5 to 12 organisms per chamber confirmed that organism density had a pronounced influence on growth during a 10-d test period. Initial attempts to remedy this by increasing the food ration showed that increasing the ration did increase growth, but simultaneously caused depression in the dissolved oxygen levels. Because the standard diet of blended flake fish food (e.g., TetraMin®) is a very fine suspension and is slow to settle out of the overlying water, we examined an alternative preparation of the food. For this food, dry flakes are first passed through a #50 sieve and then gently mixed into water these fine flakes settle readily to the bottom of the chamber and are less likely to be re-suspended during water renewal. Tests with the fine flake preparation showed that high rates of feeding still lead to reduced dissolved oxygen levels, but the fine flakes did provide greater midge growth using less food. In another series of experiments evaluating different patterns of increased rations during the 10-d tests brought us to recommend a "ramped" feeding regime of 6 mg on day -1, 2 mg per chamber on test days 0 to 3, 4 mg on days 4-6, and 6 mg on days 7-9. Under this type of regime, midge performed as well or better with less total food (e.g., 44 mg versus 66 mg). This ration was shown to be suitable for both sediment and water-only exposures. To evaluate whether changing the diet would influence results of sediment toxicity tests, we tested several field sediments having a range of contaminants and toxicity using both blended and fine flake suspensions. We found that the food preparation technique had no discernible effect on organism performance in these exposures. With the fine flake food, we can add lower amounts of food, which in turn can help maintain the overlying water quality. *This abstract does not necessarily reflect US EPA policy.*

**WP070 Compilation of historic USGS Columbia Environmental Research Center sediment toxicity and chemistry data** C.D. Ivey, U.S. Geological Survey / Columbia Environmental Research Center, US Geological Survey / Toxicology; J. Field, Office of Response & Restoration, NOAA / Assessment and Restoration Division, Office of Response & Restoration, NOAA / Coastal Protection and Restoration Division, NOAA / Office of Response & Restoration; C.G. Ingersoll, USGS / Columbia Environmental Research Center, U.S. Geological Survey / Columbia Environmental Research Center; N.E. Kemble, US Geological Survey / Columbia Environmental Research Center, USGS – Biological Resources Division / Columbia Environmental Research Center; D. MacDonald, MacDonald Environmental Sciences Ltd.; D.R. Mount, US Environmental Protection Agency / ORD; D. Smorong, Maven Environmental; T.W. Valenti, Syngenta Crop Protection LLC / National Research Council, National Research Council / U.S. Environmental Protection Agency. Using eighty-nine studies conducted at the US Geological Survey Columbia Environmental Research Center dating from 1988 to 2008, we have developed a historic sediment database comprised of matching sediment chemistry and sediment toxicity data generated with either field-collected sediments or with laboratory-spiked sediments. Contaminants evaluated in these sediments include metals, polycyclic aromatic hydrocarbons, polychlorinated biphenyls, and various pesticides. This database is currently comprised of replicate and treatment toxicity data for the following species: *Hyalella azteca* 10-day exposures (amphipod, HA10; 628 replicates), and *Hyalella azteca* 28-day exposures (HA28, 5455 replicates), *Chironomus dilutus* 10-day exposures (midge, CD10; 1298 replicates), *Lampsilis siliquoides* 28-day exposures (fatmucket mussel, FM28; 204 replicates). The database also includes individual length measures of surviving test organisms (FM28: 1,847; HA10: 4,376; HA28: 40,637). Endpoints for each species include survival, growth (as length and/or weight), and biomass. Starting lengths and weights for each species of each study have also been included in the database. Efforts are ongoing to update the database to include sediment toxicity data from studies conducted from 2008 to the present (comprising about 28 additional studies and 2,500 additional replicates). Using this database we plan to evaluate control



performance and starting and ending size of test organisms to help inform planned revisions to standard ASTM or USEPA sediment toxicity methods. We also plan to evaluate responses of test organisms in reference sediments (e.g., low in contamination) for use in calculation of reference envelopes to predict toxicity in sediments. The database will also be used to further evaluate and refine sediment quality guidelines for freshwater sediments (e.g., updating SQGs developed by MacDonald et al 2000).

**WP071 Differences in life history characteristics among different clades of *Hyalella azteca* from laboratory and field populations** K. Major, / Illinois Natural History Survey; D. Soucek, Illinois Natural History Survey / Illinois Natural History Survey, Illinois Natural History Survey / Illinois Natural History Survey, Institute of Natural Resource Sustainability / Illinois Natural History Survey; R. Giordano, University of Illinois at Urbana-Champaign / Illinois Natural History Survey. *Hyalella azteca* has been known to be a species complex for more than 20 years, and several studies have shown that extensive population-level genetic variability exists among laboratory cultures. Yet, it remains unknown the extent to which toxicology laboratories are using the same "species," or if the species cultured in laboratories can be found in Mid-Western surface waters. We sequenced the entire mitochondrial cytochrome *c* oxidase subunit I gene for *H. azteca* collected from 22 field sites in the US and Canada and 15 laboratory populations, and our analyses yielded six distinct clades. Each of the six clades exhibited high across-clade pairwise divergence (20-25%), indicating that all clades were different provisional species from one another. Most US and Canadian laboratory cultures contained members of the same species, although one Canadian laboratory housed a separate species. Further, most of the individuals from field collected populations were different provisional species from either laboratory type. We then quantified the life history characteristics of populations from two laboratory and two wild provisional species using a 42-day chronic water-only toxicity test design, and growth, biomass, and reproductive rate differed by provisional species. Given the genetic and life history characteristic differences that occur on a clade-basis, laboratories using separate provisional species to perform toxicity tests should not directly combine results for the protection of aquatic life. Further, the effectiveness of using *H. azteca* laboratory populations to act as surrogates for wild populations of this species complex is called into question.

**WP072 Precise Control of Synthetic Sediment Characteristics – Texture and Cohesion** A.M. Gonzalez, Envionyx Environmental Technologies / Environmental Services, Independent Research & Development / Environmental Services. Some in the field of sediment quality assessment emphasize field-based evaluations (e.g., rapid bioassessment protocols, in situ bioassays), while others work to improve laboratory protocols (e.g., bioassays) in relevance to field conditions and statistical power. Identifying appropriate control sediment for laboratory assessments is not trivial because natural sediment is such a complex and dynamic medium. While using locally collected natural sediment as a "reference" treatment could improve statistical power in some aspects, it could contribute to lower statistical power in others; the net effect is somewhat uncertain. Formulated sediment has been touted as a solution to this dilemma, providing a better characterized and more stable control material; however, current recipes suffer from poor realism and could contribute to poor statistical power for different reasons. The author's current work is focused on developing realistic synthetic sediment that replicates the complexity of natural sediment. Success likely will require highly specific methods and materials that can be adapted and manipulated to "fine tune" synthetic sediment properties and replicate finer levels of detail of natural sediment properties. One such property is sediment texture, which includes not only bulk properties like porosity and solids concentration, but also micro-properties like cohesiveness and plasticity. Sediment texture is what controls factors like erosion-potential, benthos burrow structure, and plastic/liquid limit indices. This presentation reports on a protocol that allows one to prepare synthetic sediment with any desired/required degree of sediment texture, ranging from extremely fluid "muck," to stable and cohesive, to extremely compact and dense. It shows that the same total mass of specific types and sizes of components (< 100  $\mu$ m particle diameter; i.e., fine sand and below) yield materials with entirely different texture properties merely by adjusting their relative proportions. A summary ternary diagram graphically depicts the range of particle combinations and proportions needed to replicate any level of sediment cohesion. Results from hexagenia exposures to synthetic sediment with varying textures demonstrate the effect.

This protocol is one of several used to create synthetic sediment that replicates natural sediment with a high degree of precision and consistency.

**WP073 Toxicity of Fungicide Formulations and Active Strobilurin Ingredients to *Hyalella azteca*** S.A. Morrison, S. McMurtry, L. Smith, J.B. Belden, Oklahoma State University / Department of Zoology. Fungicide application rates on corn have increased across the United States. As a result, contamination of adjacent aquatic systems can occur through spray drift, unintentional direct spraying, or field runoff. In order to investigate the potential toxicity of the fungicides, the epibenthic aquatic amphipod *Hyalella azteca* was exposed to two common fungicide formulations Headline® and Stratego® and their individual active ingredients. Water-only exposure studies for both formulations and active strobilurin ingredients resulted in LC<sub>50</sub> values of 17 (14-20) for Headline®, 22 (17-28) for pyraclostrobin, 26 (23-29) for Stratego®, and 30 (21-44)  $\mu$ g/L for trifloxystrobin. Exposure to Headline® formulation resulted in higher mortality than the active ingredient alone ( $p < 0.05$ ); whereas, there was no difference between Stratego® and its active strobilurin ingredient. In sediment/water microcosms, where the overlaying water was sprayed with Headline®, the LC<sub>50</sub> was 85 (59-134)  $\mu$ g/L. However, in other sediment/water microcosms where the sediment was sprayed with the same mass of fungicide 24 hr before the addition of water, no toxicity was noted even at the highest concentration tested (284  $\mu$ g/L – pesticide mass in system / water in system). LC<sub>50</sub> values for water exposure are below environmental concentrations that would likely occur following a direct overspray event into water (150 and 74  $\mu$ g/L for Headline® and Stratego® respectively). However, sediment in the system may ameliorate some of the toxicity of pyraclostrobin, especially if exposure to the wetland occurs prior to an inundation event.

**WP074 Contaminants of emerging concern (CECs); why the big fuss in California?** D. Gregorio, State Water Resources Control Board – CA; A. Heil, Los Angeles County Sanitation Districts; K. Kimbrough, NOAA; M.D. Sedlak, San Francisco Estuary Institute; K.A. Maruya, Southern California Coastal Water Research Project, SCCWRP. Although thousands of chemicals can now be detected in the environment, only a very small percentage (~200) are currently regulated and/or routinely monitored in drinking, recycled and receiving waters in California. The much larger group of unmonitored chemicals, which are herein referred to as chemicals of emerging concern (CECs) and include pharmaceuticals, personal care products, household and industrial chemicals, current use pesticides and steroid hormones, may originate from a wide range of point and non-point sources. With the increase in demand and use of recycled water, California is in the process of adopting and implementing a policy that requires monitoring of CECs to ensure protection of human health, the primary direct beneficiary of this resource. Upon discharge to receiving waters (e.g. in treated municipal wastewater effluent and/or stormwater), CECs that are readily soluble in water will remain in the dissolved (aqueous) phase and provide a route of exposure to aquatic life. A smaller subset of CECs that are hydrophobic will associate with particles, where they may remain suspended in the water column or accumulate in sediments and ultimately in tissues of aquatic and terrestrial biota. The larger concern is that exposure to CECs in water, sediment and tissue may affect wildlife as well as humans.

**WP075 Alternatives to the Fish Early Life-Stage Test: Developing a Conceptual Model for Early Fish Development** M.R. Embry, ILSI Health & Environmental Sciences Institute (HE, ILSI Health and Environmental Sciences Institute, ILSI Health & Environmental Sciences Institute (HESI) / Senior Scientific Program Manager; D.L. Villeneuve, U.S. EPA / National Health and Environmental Effects Research Laboratory, U.S. EPA; D.C. Volz, University of South Carolina, University of South Carolina / Department of Environmental Health Sciences; H. Aladjov, International QSAR Foundation; G.T. Ankley, US EPA / Office of Research and Development, National Health and Environmental Effects Research Laboratory, Mid-Continent Ecology Division, U.S. EPA / National Health and Environmental Effects Research Laboratory; S.E. Belanger, The Procter & Gamble Company / Environmental Stewardship Organization, The Procter & Gamble Company / Central Product Safety Department; K.M. Crofton, ORD, US EPA / Integrated Systems Toxicology Division, NHEERL; D. Hinton, Duke University; M.W. Hornung, U.S. EPA / National Health and Environmental Effects Research Laboratory, U.S. EPA / Mid-Continent Ecology Division; T. Hutchinson, Centre for Environment, Fisheries, and Aquaculture Sciences (CEFAS); T. Iguchi, National Institute for Basic Biology; R.



Johnson, U.S. Environmental Protection Agency / ORD/NHEERL/MED; M. LEONARD, l'OREAL; D.R. Mount, US Environmental Protection Agency / ORD; T.J. Norberg-King, U.S. EPA / ORD, NHEERL, Mid-Continent Ecology Division, US EPA / Mid-Continent Ecology Division; L.S. Ortego, Bayer CropScience / Ecotoxicology, Bayer CropScience; S. Padilla, U.S. Environmental Protection Agency / Neurotox; R.L. Tanguay, Oregon State University / Environmental & Molecular Toxicology, Oregon State University / Department of Environmental & Molecular Toxicology; J.E. Tietge, US EPA / Office of Research and Development, National Health and Environmental Effects Research Laboratory, Mid-Continent Ecology Division, U.S. EPA / Mid-Continent Ecology Division; L. Truong, Oregon State University / Department of Environmental and Molecular Toxicology, Environmental Health Sciences Center, The Oregon Nanoscience and Microtechnologies Institute and the Safer Nanomaterials and Nanomanufacturing Initiative; G. Veith, International QSAR Foundation; L.C. Wehmas, Oregon St. University / Mid-Continent Ecology Division; G. Whale, SHELL. Chronic fish toxicity is a key parameter for hazard classification and environmental risk assessment of chemicals, and the OECD 210 fish early life-stage (FELS) test is the primary guideline test used for various international regulatory programs. There exists a need to develop alternative methodologies to estimate fish chronic toxicity that use less animals and are more resource-efficient. The HESI Animal Alternatives in Environmental Risk Assessment Committee has initiated work in this arena, utilizing adverse outcome pathways (AOPs) to inform the development of alternative approaches. The Committee convened a May 2012 workshop to identify and discuss the scope of AOPs relevant to the FELS, which resulted in the development of a research strategy that will provide the conceptual and scientific foundation for the development of predictive alternative assays for chronic fish toxicity. A first step in the research strategy is to characterize and annotate FELS AOPs. Workshop participants assembled a generalized conceptual model of key anatomical and physiological developments during the embryonic and eleutheroembryonic stages of fish development. The model served as a useful foundation for sub-dividing fish development into key events as a basis for examining the underlying biology and beginning to outline putative AOPs. Further, the model provides a foundation for using exposures during critical windows of development and/or effect observations over the course of development to aid identification of chemical mode(s) of action. This poster will present the preliminary conceptual model and identify opportunities for collaborative research within the developmental biology and toxicology communities. The contents of this abstract neither constitute nor reflect official US EPA policy.

**WP076 Progress Towards the Development of a Fathead Minnow Embryo Test and Comparison to the Zebrafish Embryo Test for Assessing Acute Fish Toxicity** J.M. Rawlings, The Procter & Gamble Company / Central Product Safety; S. Bohler, University of Heidelberg / Department of Zoology; M.K. Sellin-Jeffries, A. Stultz, Miami University / Department of Zoology; J.T. Oris, Miami University / Department of Zoology, Miami University / Office for the Advancement of Research & Scholarship; T. Braunbeck, University of Heidelberg / Department of Zoology; T.J. Norberg-King, U.S. EPA / ORD, NHEERL, Mid-Continent Ecology Division, US EPA / Mid-Continent Ecology Division; S.E. Belanger, The Procter & Gamble Company / Environmental Stewardship Organization, The Procter & Gamble Company / Central Product Safety Department. The Zebrafish Embryo Test (ZFET) for acute fish toxicity is a well developed method nearing adoption as an OECD Test Guideline. Early drafts of the test guideline (TG) envisioned a suite of potential test species to be covered including zebrafish, fathead minnow, Japanese Medaka, rainbow trout, and potential marine species such as sheepshead minnow. However, during TG discussions, it was also clear that zebrafish was most well studied, clear recommendations on all key aspects were sufficient for guideline development and many laboratories were using the ZFET. All other species lagged behind. Experience with ZFET formed the foundation for new progress to develop a similarly robust fathead minnow guideline that may find utility in geographies that commonly use this species. In this poster we review key developments regarding method details on developmental milestones, time to first feeding, influence of temperature, and a limited set of information on comparative sensitivity using reference compounds and other chemicals. In general, breeding and subsequent harvesting of eggs is somewhat more time intensive and variable with fathead minnow. The time to hatch at equivalent environmental temperatures is approximately a day longer for fathead minnows. Transition from endogenous (yolk sac) to exogenous feeding

(procurement of external food items) that marks the end of the eleutheroembryo stage is somewhat later in fathead minnow. Fathead minnows are in the eleutheroembryo life stage minimally for 24 h post hatch and as long as 42 h post hatch depending on the time to hatch (fathead embryos that hatch later tend to feed exogenously earlier). Comparative toxicity using the model compound 3,4-dichloroaniline (DCA) suggests fathead minnows are approximately 3 times more tolerant to DCA than zebrafish. A limited comparative data set indicates that sensitivities are largely overlapping with neither species being uniquely tolerant or sensitive. It is our opinion that a draft SOP and addendum to the ZFET TG could be developed for fathead minnow at this time which could form the basis of a limited ring trial.

**WP077 Reducing uncertainty in species sensitivity distributions with interspecies toxicity estimation models** J.A. Awkerman, U. S. Environmental Protection Agency / Gulf Ecology Division, US EPA; S. Raimondo, United States Environmental Protection Agency / Gulf Ecology Division, National Health and Environmental Effects Laboratory, US EPA / Gulf Ecology Division; C.R. Jackson, US EPA; M.G. Barron, US EPA / Gulf Ecology Division. Determining harmful levels of contaminants for a wide range of species is limited by available toxicological data. Ecological risk assessments typically depend on empirical data from only a few species that represent a narrow range of the life history spectrum. Extrapolation models such as Interspecies Correlation Estimation (ICE) models estimate unknown toxicity values to an untested species from a well-tested surrogate, increasing the utility of available data. Developing species sensitivity distributions (SSD) with ICE-estimated toxicity values further extends data applicability by demonstrating cumulative proportion of toxicity for many species to a certain chemical. The hazard concentration HC5 represents LC50 lower than that of 95% of the species represented in the SSD. Estimation methods introduce uncertainty, such that error propagation should be addressed in extrapolation models used for risk assessment. Here we determine the effect on HC5 estimation of toxicity estimation, ICE model robustness, and surrogate species commonly used in risk assessment. We examine the potential tradeoff of using extrapolation models to increase sample size within an SSD and the subsequent introduction of uncertainty in HC5 estimates. In general, multi-surrogate ICE SSD provide recommended taxonomic proximity between surrogate and predicted species for toxicity estimation while increasing the species diversity represented within an SSD.

**WP078 A Two-Tiered Analytical Approach for Testing Contaminant Mixture Interactions** K. Callinan, University of California, Davis / Vet Med: Anatomy, Physiology and Cell Biology; L. Deanovic, University of California, Davis; S. Fong, Central Valley Regional Water Quality Control Board; S. Teh, University of California, Davis. The study of contaminant effects in mixtures is a growing field of research, and the statistical and analytical methods applied in this field do not always agree. The two theoretical models of additive response for mixtures are the model of Concentration Addition and the model of Independent Action. These two models are often compared against empirical toxicity data to determine whether chemicals act additively as predicted by their mechanisms of action, or are otherwise antagonistic or synergistic. This approach is useful for visualizing and characterizing the specific interaction or additive nature of a contaminant mixture, however it is not statistically substantiated. Statistical approaches for toxicant mixture analysis are ANOVAs, t-test comparisons, multiple logistic regressions or generalized linear model analyses. These analyses are useful for their statistical robustness, however results only indicate whether interactions are significant, but are not conclusive as to whether a mixture is additive, and by which model. By employing a unique experimental design, the current study draws on a two-tiered analytical approach, which incorporates both model comparisons as well as statistical analyses. Multiple binary mixtures of aquatic contaminants, including pyrethroid pesticides, chlorpyrifos, copper and ammonia, were tested for toxic effects and interactions on the epibenthic amphipod, *Hyalella azteca*. Concentration responses were tested concurrently in each experiment, along with six equipotent and six non-equipotent mixture treatments. Five replicates of ten amphipods were tested, and mortality, swimming behavior and growth were measured upon test termination after 10 days of exposure. Significant toxicant effects and interactions were analyzed using generalized linear model statistics, and empirical data were compared to effects predicted by the two theoretical models of mixture response. Results obtained both characterized and statistically quantified the specific interactions occurring among common aquatic contaminants. In comparison, previous studies using the same experimental

methods and test chemicals, but alternative analysis techniques had conflicting results. A statistically substantiated analysis combined with mechanistic comparisons, to confirm and further define the toxicity of a given mixture, provides a robust and comprehensive characterization of mixture toxicity in order to best predict the consequent effects on resident species.

**WP079 The Dose, Not the Concentration, Makes the Poison – Aquatic Toxicity Under Changing Water Concentrations** P.F. Landrum, Southern Illinois University-Carbondale / Fisheries and Illinois Aquaculture; D.S. Page, Bowdoin College / Chemistry; P.M. Chapman, Golder Associates; J.M. Neff, Neff & Associates. Dynamic exposures are the norm for field exposures and studies attempting to simulate field conditions. Using theoretical and case studies, we review dynamic contaminant exposures to aquatic organisms to investigate variables important for interpreting toxicity. Because the magnitude and timing of peak absorbed concentration changes with exposure dynamics, interpreting the toxic response is generally limited to the specific experiment. Extrapolation to other experiments with different exposure dynamics, or to the field where exposure dynamics can vary, requires substantial information about toxicokinetics and exposure dynamics along with temporal threshold data for extrapolation between studies. This is particularly true for mixture exposures, where the concentration and composition and, therefore, the timing and magnitude of exposure to individual components of different potency can vary. For the short term, initial water concentration can be considered a conservative measure of exposure, although the extent of conservatism will vary with the dynamics of exposure and the toxicokinetics of the chemicals of interest. A better metric for interpreting toxicity is the peak absorbed dose, although this neglects toxicodynamics and requires temporal measures of accumulated dose so that the peak concentration can be determined. Aquatic toxicology studies are needed that develop temporal thresholds for absorbed toxicant doses. Such studies would allow for better extrapolation between conditions of dynamic exposure. Improved experimental designs are also needed, including high quality temporal measures of both the exposure and the absorbed dose.

**WP080 The relevance of phylogenetic modelling to ecotoxicology: an example involving linear solvation energy parameters to predict multiple tolerances** G. Guenard, Université de Montréal / Département des sciences biologiques; P.C. von der Ohe, Helmholtz-Zentrum für Umweltforschung / Department of Effect Directed Analysis; P. Legendre, Université de Montréal / Département des sciences biologiques. We present an approach to build statistical models of species tolerance to chemical substances using phylogenetic information together with chemical properties, namely the parameters of linear solvation energy relationships (LSER). Trait variation is often structured with respect to phylogeny; with traits bearing the traces of the evolutionary processes that shaped them. Methods now exist to use phylogenetic signals to extend the empirical knowledge about traits that we only have for a limited number of species (for instance, because the trait is difficult to estimate) to a larger set of species. The ability of species to tolerate adverse environmental stressors is an important feature to assess their continued existence in the environment. In most practical situations, multiple stressors simultaneously affect many species in ecosystems. Hence, knowing the tolerances of sufficiently large number of the species towards a sufficiently large number of stressors require considerable effort and time. To simplify that task, we propose to use an analytical framework whereby phylogenetic eigenvector maps (ie. a spectral decomposition methods for phylogenetic signals) are used within a bilinear model (ie. a multiple-response linear models with two tables of explanatory variables). To illustrate the method, we used components of the phylogenetic signals of median lethal concentration ( $LC_{50}$ ; 48h) for 22 species, with five LSER parameters describing twelve common chemicals, in a single bilinear model. The resulting model describe a substantial fraction of the variation ( $R^2_{adj} = 0.73$ ) in the log-transformed  $LC_{50}$ . Overall, 85% of predicted  $LC_{50}$  values deviated from observed values by a factor of ten or less in cross-validated models, a situation observed for eight compounds out of twelve.

**WP081 Estimating Confidence in Toxicity Predictions** M. Lawless, A. Lee, J. Zhang, D. Zhuang, R. Fraczkiwicz, M. Waldman, R. Clark, Simulations Plus, Inc.. How can one estimate the overall reliability of a model's predictive accuracy? We have attempted to do this for ensembles of binary artificial neural network classification models (ANNs) using "ensemble confidence" and weighting the observations. The models were built for two endpoints: reproductive toxicity (60%:40%, nontoxic:toxic)

and skin sensitization (20%:80% nonsensitizer:sensitizer), representing balanced and unbalanced data sets, respectively. The individual models in each ensemble are optimized independently and share a common architecture, but each uses a different split of the data between ANN training and verification sets, while the external test set remains the same. Positive and negative "votes" from the constituent models are then tallied with majority rule determining the overall prediction. Confidence can be expressed as a simple percentage of positive or negative votes, but that approach does not fully reflect the effect of increasing the ensemble size on confidence. As an alternative approach, the "ensemble confidence" can be computed for each prediction using the binomial distribution based on the majority vote. We also explore the effect of differentially weighting positive and negative observations during model training and evaluate the confidence estimates. Ensemble confidences for the compounds in the training and test sets can help gauge the overall quality of the ensemble model and reliability of predictions for external compounds.

**WP082 Comparative Probabilistic Hazard Assessment of CYP Activity for Organophosphates** A.M. Parr, Baylor University / Environmental Health Science Program; K.A. Connors, B.W. Brooks, Baylor University / Department of Environmental Science. Though tens of thousands of chemicals are used around the world, very little toxicity data exists for many of these chemicals. To address this need, regulatory agencies are increasing data requirements for existing and new chemicals. In the United States, the U.S. Environmental Protection Agency's (EPA) TOX21 program is attempting to fill data gaps related to toxicity pathways and other activities of industrial contaminants. Such information is necessary for developing predictions of toxicological activities of other unstudied chemicals with common characteristics. Our research group has recently employed probabilistic hazard assessment (PHA) with chemical toxicity distributions (CTDs) to predict the likelihood of encountering toxicity from chemicals sharing common modes of action and residing within the same class. We extended this approach in the present study to perform a novel evaluation of organophosphate (OP) insecticide induction of several cytochrome (CYP) P450 enzymes. We specifically performed comparative PHAs of EPA TOXCAST datasets for five cytochrome P450 enzyme assays (CYP 1A1, 1A2, 3A4, 2B6, 2C9), which were performed for 8 common OPs over 48 hr exposure periods. We predicted that there is a 30.5% (CYP 2B6), 24.3% (CYP 2C9), 21.8% (CYP 3A4), 5.2% (CYP 1A2), and 0.0035% (CYP 1A1) probability of encountering an OP that will induce various P450 activities at concentrations below 1  $\mu$ M. Based on this analysis, OPs are most likely to induce CYP 2B6 activity, whereas CYP 1A1 induction by OPs is not anticipated.

**WP083 High-Throughput Cell-Free Neurochemical Screening Assays to Predict Adverse Outcomes in Fish, Mammals, and Birds** N. Basu, University of Michigan / Department of Environmental Health Sciences; University of Michigan / Department of Environmental Health; D. Nam, Chonnam University; J. Head, University of Michigan; C. Murphy, Michigan State University. Cell free, in vitro bioassays have been developed in biomedicine to screen, for example, neurotoxic agents and novel pharmaceuticals. Cell-free methods are now a component of the U.S. EPA's ToxCast program, and development of such bioassays for predicting adverse outcomes is particularly attractive in ecological risk assessment owing to the lack of screening methods that span several taxa and a limited ability to conduct whole-animal bioassays. Here we describe a series of in vitro assays that may be used to assess whether contaminants interact with, and possibly disrupt the function of, various neurotransmitter receptors and enzymes that mediate reproduction and behavior across fish, birds and mammals. In this presentation, we focus specifically on a series of metals ( $Hg^{2+}$ ,  $CH_3Hg^+$ ,  $Pb^{2+}$ , total Se,  $Sn^{2+}$ ,  $As^{3+}$ ,  $Cd^{2+}$ ,  $Cr^{6+}$ ,  $Mn^{2+}$ ) to determine if they inhibit binding to the muscarinic acetylcholine receptor (mAChR) and N-methyl-D-aspartate receptor (NMDAR) in brain cortical tissues collected from several fish (goldfish, perch, lemon shark, mako shark), birds (chicken, bald eagle), and mammals (mouse, polar bear, mink, common dolphin, Atlantic white-sided dolphin). First, saturation binding curves were developed from each species to calculate mean receptor density ( $B_{max}$ ) and ligand affinity ( $K_d$ ). Next, samples were exposed to the aforementioned metals at several concentrations to derive IC<sub>50</sub> (inhibition concentration 50%) values. Based on IC<sub>50</sub> values and resulting inhibition constants ( $K_i$ ), generalized rank-order potencies for metals and species were developed and will be presented. In addition to this focused presentation on metals, we will also highlight our research using this in vitro platform to study pulp and paper mill effluents



(risks to fish) and organochlorines (risks to marine mammals). We will discuss the pros and cons of this screening platform, and also a strategy by which *in vitro* data outputs may be modeled and scaled to predict individual-based adverse outcomes and thus be used in a predictive ecotoxicological context.

#### **WP084 Toxicogenomics to group environmental chemicals *in vitro*?**

A. Hawliczek, / Centre for Fish and Wildlife Health, University of Berne / Centre for Fish and Wildlife Health; J. Legradi, J. Legler, VU University Amsterdam; H. Segner, University of Bern / Centre for Fish + Wildlife Health. In our study we assessed the suitability of integrating toxicogenomics into chemical classification. In order to make risk assessment of high numbers of chemicals feasible, chemicals have to be classified into groups, as thereby risk assessment can be done group-wise instead of substance by substance. One frequently used classification approach is to group chemicals according to their mode of action (MoA). However, a critical point in this grouping approach is the properties according to which substances can be assigned to a MoA. A classical approach for MoA assignment of chemicals is on the basis of the relationship between their toxicity *in vivo* and their structural and/or physicochemical properties. Here, we explored the potential of *in vitro* microarray data – i.e. a biologically based system – to contribute to MoA assignment of chemicals. It is known that single chemicals can induce chemical-specific gene expression profiles (barcodes). The question addressed in our study is whether barcodes can also be generated for substances sharing a common MoA and further if this can be done in an *in vitro* system. To this end, we tested uncouplers of oxidative phosphorylation (OXPHOS). Test compounds were selected based on (Q)SAR models of uncouplers as well as on the basis of biological mechanistic information. Cells *in vitro* were exposed to equipotent concentrations of the uncouplers and gene expression profiles were measured. The microarray results indicate OXPHOS specific “barcodes” for uncoupler MoA. The findings from this study point to the value of -omic approaches to verify or correct physicochemical or structural-based MoA assignment, as it is routinely used in ecotoxicological risk assessment. Further, the results show the feasibility of MoA grouping of chemicals on the basis of gene profiling using *in vitro* system.

#### **WP085 Oxygen consumption in *Daphnia magna* as a rapid bioindicator of changes in water quality**

M.C Stensberg, Purdue University / Department of Agricultural and Biological Engineering; M. Zeitchek, K. Inn, Purdue University; E. McLamore, Purdue University / 1Birck-Bindley Physiological Sensing Facility and Department of Agricultural and Biological Engineering; M.D. Porterfield, Purdue University / Birck-Bindley Physiological Sensing Facility 2Department of Agricultural and Biological Engineering; M.S. Sepulveda, Purdue University / Forestry & Natural Resources and School of Civil Engineering, Purdue University / Forestry & Natural Resources &. Currently there are a variety of analytical chemistry based water quality monitoring systems which are capable of measuring low level pollutants. However, most are not readily field deployable and in those that are, sample collection strategies are not optimized. We plan to develop a biological based detector of changes in water quality that could be used solely as a detector or used as a trigger system to be coupled with traditional analytical systems. The purpose of the presented research is to determine experimentally whether oxygen consumption in *Daphnia magna* embryos can serve as a suitable indicator of changes in water quality. For this experiment we examined an array of chemicals used in a similar experiment with fathead minnows, all of which have differing mechanism of toxicity: potassium cyanide, cadmium chloride, atrazine, malathion, and pentachlorophenol. Concentrations used were based on the EPA acute and chronic ambient water quality guidelines for the protection of aquatic life. The effects were first monitored by measuring oxygen flux at the surface of the embryos during exposure, then the experiment was scaled up to optically measuring oxygen concentration change in the microenvironment (modified 96-well plate) around the embryo to determine if the technique could be scaled to a high throughput system. All chemicals elicited a significant change in oxygen consumption in less than two hours during flux experiments, and preliminary data indicates that similar changes will be observed in the microenvironment. The results indicate that *D. magna* embryos serve as a suitable bioindicator species for rapid detection of low-levels of contaminants in water. Because of this response to this broad array of chemicals, our results imply that oxygen consumption may serve as a detector for chemicals, such as emerging contaminants, that may not have had their water quality criteria defined yet.

#### **WP086 Characterizing the Chronic Toxicity of Total Dissolved Solids on Two Freshwater Invertebrates: *Ceriodaphnia dubia* and *Utterbackia imbecillis***

K.A Johnson, Clemson University / Institute of Environmental Toxicology; S.J. Klaine, Clemson University / Institute of Environmental Toxicology (CU-ENTOX), Clemson Institute of Environmental Toxicology / Institute of Environmental Toxicology (ENTOX), Clemson University / Institute of Environmental Toxicology (ENTOX). Through human activities, such as mining operations, agricultural irrigation and power plant effluents, excess Total Dissolved Solids (TDS) can enter into freshwater systems. Total Dissolved Solids is a measure of all the ions present in solution, which contribute greatly to salinity. Freshwater organisms must use energy to maintain a homeostatic balance of ions between their bloodstream and the environment. In general, increased ionic strength results in increased stress to these organisms. However, the contribution of individual ions to this stress is unclear, and although acute toxicities have been well characterized for many ions, the chronic effects are less known. This study will use a series of 8-day chronic bioassays to characterize the varying toxicities of chloride, sulfate and bicarbonate on *Ceriodaphnia dubia* and *Utterbackia imbecillis*. Preliminary research indicates the *C. dubia* reproduction can be used to predict the 28-day growth endpoint in *U. imbecillis*. Results from this study will facilitate better management of coal-fired power plant effluents. Further, these results will contribute to the growing body of data that suggests *C. dubia* chronic endpoints are protective of freshwater mussel endpoints.

#### **WP087 Boron Chronic Toxicity to the Fatmucket Mussel *Lampsilis siliquoidea* in Water-only and Whole-sediment Exposures**

S. Hall, ENVIRON International Corporation / Ecotoxicology, ENVIRON / Ecotoxicology; R. Lockwood, ENVIRON International Corp. / Department of Ecotoxicology; M. Harrass, Rio Tinto Minerals / Manager, Env. & Health Science.. Flow-through tests are desirable to maintain water quality in chronic sediment tests. However, sediment concentrations of highly soluble materials such as boric acid will be depleted, even eliminated, in a flow-through design that lasts several days. A test design based on modifications of OECD and ASTM methods was implemented to evaluate boron toxicity (as boric acid) in chronic (21 d) flow-through tests using the Fatmucket Mussel (*Lampsilis siliquoidea*). Whole sediment was spiked with boric acid, and overlying water was also spiked at concentrations equivalent to targeted sediment concentrations. Parallel testing in a water-only format allowed comparison to spiked-sediment results. IC25 values in the spiked sediment testing were 363 mg B/kg dry weight sediment (based on mortality) and 311 mg B/kg dry weight sediment (based on biomass measured as shell growth). The measured pore-water concentrations in the sediment test were in good agreement with targeted concentrations and very similar to those in the water-only test. Toxic effects (21 d, IC25) in the water-only test were 38.4 mg B/L (based on mortality) and 34.6 mg B/L (based on biomass measured as shell growth). Toxic effects in the spiked-sediment tests were seen at pore-water concentrations corresponding to toxic effect concentrations in the water-only test, indicating that, for these mussels, toxicity is associated with the dissolved (pore-water) phase.

#### **WP088 Use of Native Fish Species from the Gulf of Mexico to Evaluate the Toxicity of Petroleum Hydrocarbons**

B.S Echols, Virginia Tech / Department of Biological Sciences, Florida International University / Southeast Environmental Research Center; A. Smith, B. Seda, G. Rand, Florida International University. Aquatic toxicity tests are predominately conducted with standard species using established test guidelines. However, these species may not be protective of more sensitive native species that are in direct contact with environmental perturbations. Native species are not routinely used in acute and chronic toxicity tests as they are often more difficult to obtain or culture, are more sensitive to handling stress, and important life history characteristics, such as larval survivorship and development are not well understood. In order to use native species of invertebrates and fish in acute and chronic testing programs, testing procedures must first be established and validated and background information on hatchability and expected larval survivorship must be determined. Native Gulf of Mexico fish species, including Spotted Seatrout (*Cynoscion nebulosus*), Red Drum (*Sciaenops ocellatus*), Red Porgy (*Pargus pargus*), and Cobia (*Rachycentron canidrum*) are currently being evaluated for use in native species toxicity tests to elucidate the toxicity of PAHs from field collected MC252 crude and weathered oil samples. The main objective of this evaluation was to ascertain species sensitivity to the oil samples and to document key life



history endpoints, including larval survivorship and development rate. During test capability development, standard reference toxicant tests (Potassium chloride) were used to compare responses between native species reported sensitivity ranges of routine standard species (*Menidia beryllina*, *Cyprinodon variegatus*). Hatchability studies were also used to establish an expected rate of larval hatching success in control water. Preliminary studies indicate issues with survival of native species may be due to the combination of insufficient feeding regimes during test duration and cannibalism. The presentation will discuss the modifications that must be made for native species within the regulatory standard methodologies for toxicity testing with fish in the laboratory. It will also include discussion of data on their responses to standard reference toxicants and how they fit within the distribution of sensitivities of standard laboratory test species.

**WP089 Bioavailability of Metals in Soil: Considerations for Ecological Risk Assessment**

**A. Bailey**, SLR International Corporation; **M. Steljes**, SLR International Corporation / Risk Assessment and Toxicology. Bioavailability of metals is an important consideration in ecological risk assessment. Metals in soil are often assumed to be 100 percent bioavailable and the total amount detected is used in estimating the daily dose to exposed receptors. Many metals, however, are often present in soil in an insoluble form, greatly reducing the amount available for uptake across the gut. This can have important impacts on exposure to ecological receptors and quantification of risks, similar to that observed for lead in humans. Outside of lead and arsenic, few studies are available that attempt to mimic gut absorption of metals in mammals. Review of the laboratory model bioavailability literature indicates there is a general linear trend between bioavailability and fluid-to-solid ratio, and a general inverse relationship between bioavailability and pH. This study attempted to mimic the range of stomach acid pH values, food-in-stomach residence times, and gastric fluid-to-solid ratios in the stomach of wildlife receptors often used as indicator species in ecological risk assessments to provide an estimate of potential barium bioavailability. Total and leachable barium, as well as total organic carbon, were analyzed in soil samples from locations with barium originating from either background or contaminated sources. Using the synthetic precipitation leaching procedure (SPLP) modified to a pH of 2.5, residence time of 8 hours, and a fluid-to-solid ratio of 20:1, the bioavailable percentages of barium estimated in soil samples ranged from 0.3 to 6.2 percent. Results were similar across sample groups. These results indicate that barium bioavailability is much lower than the 100 percent often assumed in ecological risk assessments, and that consideration of metals bioavailability may have important impacts on ecological risk estimates and potential costs associated with site remediation.

**WP090 A Multi-Agency Approach to Measuring the Public Health Burden of Harmful Algal Blooms**

**D.A Perkins**, C. Hedman, University of Wisconsin – Madison / Wisconsin State Laboratory of Hygiene; **G. LaLiberte**, Wisconsin Department of Natural Resources; **E. Wollenburg**, **M. Werner**, Wisconsin Division of Public Health. Harmful algal blooms are an annual problem which impacts the use of Wisconsin's most popular recreational waters. The Wisconsin Division of Public Health, the Wisconsin Department of Natural Resources and the Wisconsin State Laboratory of Hygiene are working together to measure the public health burden of harmful algal blooms (HABs) and to better characterize environmental conditions during these blooms. Between 2009 and 2011, the Wisconsin Division of Public Health received 100 complaints of human and animal illness associated with exposure to HABs. Analysis of environmental data identified microcystin-LR and anatoxin-A as the most common algal toxins in Wisconsin waters. Air monitoring along lake shorelines has also indicated elevated levels of ammonia and hydrogen sulfide, which may contribute to respiratory symptoms reported by lakeshore residents. In addition to collecting health and water quality data, this surveillance program has also expanded outreach to veterinarians, local lakes associations and the general public. The goal of this outreach effort is two-fold; to increase reporting of illnesses related to HABs and to increase awareness of the potential hazard associated with algal blooms. Current surveillance and outreach efforts will enhance our understanding of the public health burden of HABs and facilitate the development of prevention measures aimed at reducing exposures to harmful algal blooms.

**WP091 Influence of Land Use on Cyanotoxins in Small Water Bodies of South-Central Saskatchewan**

**A. James**, University of Saskatchewan / Toxicology; **J. Davies**, Saskatchewan Watershed Authority; **J. Schoenau**,

University of Saskatchewan / Agriculture; **J.E. Naile**, U.S. Environmental Protection Agency / Ecosystems Research Division; **M. Wickstrom**, University of Saskatchewan / Toxicology Centre. Blooms of cyanobacteria that produce toxic compounds occur in freshwater ponds and wetlands in Saskatchewan, where they pose a risk to domestic livestock and wildlife. Most documented poisonings of animals that are caused by cyanotoxins result from exposure to microcystins, which cause damage to the liver. The present study was intended to identify whether agricultural land use and/or geographic region were associated with the presence and abundance of toxins produced by cyanobacteria in smaller Saskatchewan water bodies in the Saskatchewan northern prairie ecozone. Additionally, this study was also intended to determine if concentrations of nutrients in surface waters and/or soils were different for small water bodies and their surrounding drainage basins located within arable land compared with permanent pasture. Study sites were clustered in three regions of southern and central Saskatchewan, and included Saskatoon/Aberdeen, Elrose/Rosetown and Swift Current. These regions were located within two of three main soil zones found within southern Saskatchewan. Each cluster consisted of eight small water bodies located within arable crop fields, and eight small water bodies located in permanent pasture land used for cattle grazing. Microcystins, which are cyanobacterial hepatotoxins, were identified and quantified by use of both HPLC-MS/MS and a bioassay that measured inhibition of phosphatase activity. Microcystins were detected in water bodies located within areas of both types of land-use. However, the water bodies surrounded by arable land contained significantly greater concentrations of these toxins, suggesting that practices specific to arable land are influencing abundances of microcystins. Concentrations of soluble reactive phosphorus (in water) and total phosphorus (in water and soil) were found to be significantly different between land uses, and were positively correlated with concentrations of microcystins in water. Nitrogenous compounds in soil and water were also positively associated with concentrations of microcystins. This result suggests that repeated fertilization with nitrogenous fertilizers is possibly leading to accumulation of nitrogenous compounds in soil, which can be mobilized into local surface water, and potentially promote the growth of cyanobacteria.

**WP092 New Insights into the Adverse Outcome Pathway for Domoic Acid in Aquatic and Terrestrial Organisms**

**I. Schultz**, Battelle Pacific Northwest National Laboratory / Marine Science Laboratory, Ecotoxicology Group, Battelle Northwest Laboratories; **C. Timchalk**, **K. Minard**, **D. Woodruff**, Battelle Northwest Laboratories; **K. Watanabe**, Oregon Health Sciences University. Domoic acid (DA) is an excitatory neurotoxic amino acid produced by several marine algal species and is the causative agent of amnesic shellfish poisoning in humans, seabirds and marine mammals. DA is a potent agonist for kainate receptors (ionotropic glutamate receptors whose activation leads to the influx of Na<sup>+</sup> and Ca<sup>2+</sup>). Increased Ca<sup>2+</sup> concentrations result in neuronal excitotoxicity and cell death primarily in the hippocampus, which produces seizures, impairs learning and memory, and alters behavior in some species. An adverse outcome pathway (AOP) is a conceptual sequence of events beginning with a molecular-level initiating event that triggers a cascade of reactions resulting in an adverse outcome upon a population. Development and implementation of an AOP model requires knowledge of contaminant dosimetry along with estimation of dose-dependent perturbations across varying biological scales (cells→organs→systems→organism→populations). In vitro, cell-based assays are expected to provide the bulk of experimental data for AOP models while computational modeling is used to extrapolate across biological scales. A conceptual AOP model for DA was recently described and suggested cellular assays of Ca<sup>2+</sup> regulation could be developed to assess impacts of DA toxicity. However, important challenges identified for implementing a DA / excitatory neurotoxin AOP is the lack of information on interpreting the relationship between neuronal cell death, hippocampus function and behavior. For algal toxins like DA, which are biomagnified in aquatic food webs, there is also a lack of mechanistic understanding on interspecies differences in bioaccumulation, particularly with respect to bioavailability and disposition. In this presentation, we will summarize our current understanding of DA toxicokinetics and the physiological mechanism(s) that contribute to the unusual patterns of bioaccumulation described in diverse invertebrate and vertebrate species. With respect to hippocampus function, new imaging technologies are becoming available that better allow integration of dosimetry and response data within a 2- and 3-D space at the organ / organ system scale. Examples to be discussed and their application

to the excitatory neurotoxin AOP are Magnetic Resonance Imaging and Spectroscopy, Positron Emission Tomography and various types of Mass Spectrometry Imaging.

**WP093 Response of immunomodulatory NF- $\kappa$ B signaling pathway to microcystin-LR exposure in HepG2** J. Zhang, J. Chen, Zhejiang University / Institution of Environmental Sciences; Z. Xia, Zhejiang University / Institution of Life Science. Regional cyanobacteria blooming has resulted in the contamination of microcystins in China, which are specific hepatotoxins having shown the potential to pose a series of human health risks. The nuclear transcription factor NF- $\kappa$ B is important for immune and inflammatory responses and its misregulation has been linked to cancer, inflammatory and autoimmune diseases. Activation of NF- $\kappa$ B involves sequential phosphorylation, ubiquitination and proteasomal degradation of the inhibitory subunit I $\kappa$ B $\alpha$ . Little is known about the process and mechanism of microcystin-LR-induced NF- $\kappa$ B activation, while NF- $\kappa$ B signaling pathways involves a series of kinases and phosphatases which could be influenced by the phosphatase inhibitory activity of MC-LR. In this study we investigated the effect of MC-LR exposure on NF- $\kappa$ B activation by using human hepatoma cell line HepG2. 2-days' exposure of HepG2 cells to MC-LR ranging from 1-1000  $\mu$ g/L didn't affect cell viability by MTT assay. In the *in vitro* cell-free-based NF- $\kappa$ B activation assay, MC-LR induced I $\kappa$ B $\alpha$  hyperphosphorylation in a dose-dependent manner. In the luciferase-based NF- $\kappa$ B activation reporter assay, 500  $\mu$ g/L MC-LR induced NF- $\kappa$ B activation in a time-dependent manner. Pre-treatment of HepG2 cells with MC-LR induced a state of cellular hyperresponsiveness toward subsequent stimulation with TNF $\alpha$ , a potent proinflammatory cytokine. The implication of our results to comparative toxicology of harmful algal blooms (HABs) to human liver cancer risk and the development of human health risk based drinking water standards in China will be discussed.

**WP094 Using *Caenorhabditis elegans* (*C. elegans*) to Evaluate Microcystins (MCs) Potential Neurotoxicity** C. Moore, UC Davis; B. Puschner, University of California, Davis. Produced by cyanobacteria worldwide, MCs can contribute to multifactorial diseases in mammals, fish and plants. The primary route of exposure is through oral ingestion of contaminated surface water from lakes, rivers, ponds and estuaries. While MCs' acute hepatotoxic effects are intensively studied, there is a great need to evaluate MCs effects on the nervous system. The remarkable genetic and neuro-biochemical conservation between *Caenorhabditis elegans* (*C. elegans*) and humans make it an ideal neurotoxicity model. Recent studies show *C. elegans* have sensitive neurological endpoints after 24-hour exposure to MC-LR (one of the 80+ variants of MCs and the most commonly studied). *C. elegans* were exposed to 0-100 $\mu$ g/L of MC-LR using different exposure methods. Exposed and non-exposed adult *C. elegans* were compared. Chemotaxis indices to the odors benzaldehyde and diacetyl were used to measure behavior patterns and determine if MC-LR targets specific sensory neurons differently. Attraction to benzaldehyde is controlled by the AWC sensory neuron while the AWA sensory neuron controls attraction to diacetyl. Wildtype N2 and bah-1 worms were utilized. The bah-1 strain was more sensitive to MC-LR than wildtype worms due to its weakened cuticle.

**WP095 Addressing Watershed Scale Impacts in a Life Cycle Study of Two Redwood Lumber Production Systems** A. Foster, T. Schultz, Scientific Certification Systems. Many SETAC participants are familiar with Life Cycle Assessment (LCA), a cradle-to-grave evaluation tool used to understand the environmental and human health impacts of a product, system, or service over its entire life cycle. While ISO 14044 establishes the general framework for conducting LCAs, it does not specify the impact categories to be addressed, nor spell out specific calculation methods. As a result, LCA models have evolved without necessarily addressing the site-specific ecological impacts that are associated with the product or system being studied, and without the necessary methods to capture this important data. This presentation will provide an overview of methods now available for use within the LCA framework for capturing biome impacts, including those occurring on the watershed scale. A riveting case study will be presented that demonstrates this method. The case study is an LCA comparison of the same redwood forest managed under two different forest management practice regimes. Impacts to both aquatic and terrestrial biomes will be discussed. In addition, the case study will include a thought-provoking demonstration of how different LCA models can produce different results.

**WP096 An Integrated Approach to Assess Stormwater Impacts on Coastal Preserves: In Situ Monitoring, Bioaccumulation, and Biological Surveys in La Jolla, CA** R. Schottle, AMEC Environment & Infrastructure; C. Stransky, AMEC Earth and Environmental, AMEC Earth & Environmental / Aquatic Sciences, AMEC Environment & Infrastructure / Senior Aquatic Scientist; S. Gruber, Weston Solutions, Inc., Weston Solutions; A. Sonksen, City of San Diego / Transportation & Storm Water Department. The variable nature of stormwater runoff presents unique challenges with regard to accurately characterizing water quality and potential receiving water impacts. A multiple lines of evidence (MLE) approach is needed to more accurately characterize potential biological impacts and stressors of concern to cost-effectively identify future Best Management Practices (BMPs). This is particularly true when assessing stormwater impacts on coastal marine preserves such as Areas of Special Biological Significance (ASBS), where reliance on water quality monitoring alone may not be protective of the unique beneficial uses found in these areas. This presentation focuses in on the experimental design and results from four key lines of evidence that were used for an integrated approach to assess potential stormwater impacts in a rocky, semi-protected intertidal habitat within the La Jolla ASBS in San Diego, California: 1) *In situ* toxicity and concurrent real time salinity profiles; 2) receiving water chemistry; 3) bioaccumulation of contaminants of potential concern in native marine species; and 4) biological community assessments. Initial results of this multi-year study have shown that potential impacts to the ASBS from stormwater is highly dependent upon the inter-related dynamics of tidal stage, receiving water habitat, proximity to storm drain outfalls, and cross-contamination from discharges well outside of the ASBS. Freshwater alone appears to be more of a stressor than any associated contaminants in the receiving water. Effects are more pronounced in the upper intertidal zone where some tide pools and channels are completely fresh at low tide during rain events. Site-specific variables are important considerations when assessing potential stormwater impacts in a marine environment and thus need to be carefully considered when implementing and interpreting results from general compliance-related monitoring programs.

**WP097 Assessing the Accuracy of a Land Use-based Coho Spawner Mortality Model in Puget Sound Lowland Streams** D. Baldwin, NOAA Fisheries – Northwest Fisheries Science Center / Northwest Fisheries Science Center; J. Spromberg, NOAA Fisheries / Northwest Fisheries Science Center, NOAA Fisheries – Northwest Fisheries Science Center / Environmental Conservation Dep; B. Feist, E. Buhle, NOAA Fisheries – Northwest Fisheries Science Center; S. Damm, U.S. Fish & Wildlife Service – Washington Fish & Wildlife Office; J.W. Davis, U.S. Fish & Wildlife Service – Washington Fish & Wildlife Office / Environmental Contaminants; N. Scholz, NOAA Fisheries – Northwest Fisheries Science Center / Northwest Fisheries Science Center. Monitoring efforts evaluating urban stream restoration effectiveness in the greater metropolitan area of Seattle, Washington, have detected high rates of premature mortality among adult coho salmon (*Oncorhynchus kisutch*) in restored spawning habitats. Affected animals display a consistent suite of symptoms (e.g., disorientation, lethargy, loss of equilibrium, gaping, fin splaying) that ultimately progresses to death within a few hours. Annual rates of pre-spawn mortality (PSM) observed over multiple years across several drainages have ranged from ~20% to 90% of the total fall run within a given watershed. The current understanding of coho PSM is that it occurs when pollutants accumulate on impervious surfaces during summer and early fall dry periods are washed into coho-bearing streams by fall storm events. The phenomenon seems to require both specific land uses and precipitation patterns. Previous spatial analyses that modeled the relationships between PSM, land use and precipitation patterns, concluded that the relative proportion of local roads, impervious surfaces, and commercial property in corresponding drainage basins was most strongly correlated with coho PSM rates. Fitted models from these analyses were used to build a map of predicted coho spawner mortality throughout most unmonitored basins of the Puget Sound lowlands. As urban expansion continues, areas that once supported coho salmon may experience PSM rates that could jeopardize coho salmon population viability. In order to assess the accuracy and precision of predictive models and improve our ability to predict the fate of existing coho populations that may become imperiled given future urban growth scenarios, we regressed a compilation of coho PSM data that was not used in the aforementioned analyses against corresponding prediction values. We found that overall model prediction accuracy and precision of PSM rates



was acceptable given the size of our modeling domain and the sample size for the original PSM predictions.

**WP098 Assessment of the toxicity of sediments in a small urban creek contaminated with runoff water from a municipal fire in, Columbia Missouri** N.E. Kemble, US Geological Survey / Columbia Environmental Research Center, USGS – Biological Resources Division / Columbia Environmental Research Center; D.K. Hardesty, USGS; C.G. Ingersoll, USGS / Columbia Environmental Research Center, U.S. Geological Survey / Columbia Environmental Research Center; R. O'Hearn, Missouri Department of Conservation. About 4 million liters of water was used to treat a fire at a shopping mall in Columbia Missouri in April of 2012. Runoff of the water used to treat the fire flowed through into a storm-water drainage system and reached Flat Branch Creek (a shallow 8-km urban creek) resulting in a die off of about 14,000 fish. The fish kill was attributed to low dissolved oxygen in the creek for several days after the event. Oil and other waste materials associated with the runoff from the fire coated the surface of sediments in the creek. A likely source of the contamination was the burning of a large quantity of oil stored a facility at the shopping mall complex. Sediment toxicity was evaluated in six samples collected from the creek and in a reference sediment collected from Hinkson Creek (above the confluence of Flat Branch Creek into Hinkson Creek). A control sediment from West Bearskin Lake, MN was also tested. Whole-sediment toxicity tests were conducted with the amphipod *Hyalella azteca* (28-d exposures measuring effects on survival, weight, or biomass of amphipods). Chemicals of potential concern in the sediments likely resulting from the runoff water associated with the fire included oil, polycyclic aromatic hydrocarbons, paint, epoxy, paint thinner, brake fluid, battery acid, and other unknown contaminants. Amphipod survival was significantly reduced in all six of sediments collected from the impacted creek compared to the control sediment or the reference sediment. The sediment samples nearest the location were the runoff initially entered the creek were most toxic, with toxicity decreasing downstream to the confluence of the impacted creek with the reference creek. Growth and biomass endpoint evaluations are ongoing. Additional analyses are planned to determine potential chemicals that may have contributed to the toxicity of the sediment samples. Additional studies are also planned later this summer to determine if the toxicity and contamination are persistent in the creek. Results of this study may assist fire departments to develop improved mechanisms to protect small streams when dealing with urban fires.

**WP099 Characterizing spatial and temporal variability within watersheds in the Stream Pollution Trends (SPoT) Monitoring Program** K. Siegler, University of California – Davis / Environmental Toxicology; J. Voorhees, University of California – Davis / MPSL – Environmental Toxicology; S. Katz, University of California-Davis / Environmental Toxicology; B.M. Phillips, University of California – Davis / Environmental Toxicology, University of California-Davis / Granite Canyon; B. Anderson, L. Jennings, J. Hunt, R. Tjeerdema, University of California Davis / Environmental Toxicology. The Stream Pollution Trends (SPoT) program conducts statewide monitoring surveys as part of the Surface Water Ambient Monitoring Program (SWAMP). Sediment samples have been collected annually since 2008 at streams throughout California and analyzed for sediment toxicity and a suite of pesticides, trace metals and trace organic compounds. These data are used to evaluate long term water quality trends statewide. Sampling stations are located at the base of watersheds using a USGS NAWQA integrator site design. In order to investigate how well SPoT base-stations represent spatial and temporal variability in the watersheds, additional stations were sampled and analyzed three times per year (summer, fall and winter) throughout six different watersheds between 2010 and 2011. Toxicity and total pyrethroid concentrations (2010) were then analyzed using an analysis of variance to determine statistical differences among the samples. Toxicity results from half of the watersheds were not significantly different spatially or temporally, but base station toxicity from five of the six watersheds was significantly different from year to year (2008-2011). Watersheds with a greater incidence of toxicity demonstrated greater spatial and temporal variability. There were no significant spatial or temporal differences in the 2010 pyrethroid results. These preliminary results indicate the spatial and temporal variability was watershed specific. Although additional samples would add resolution to determining short-term trends at variable sites, a single sample per year is representative at sites with low variability.

**WP101 Evaluation of the effects of storm water and receiving water samples collected from an Area of Special Biological Significance on two marine species** S. Douglass, H. Bailey, P. Arth, Nautilus Environmental; C. Stransky, A. Cibor, AMEC E&I; R. Kolb, City of San Diego. Toxicity testing was conducted on storm water and receiving water samples associated with three different outfalls discharging into the La Jolla Area of Special Biological Significance (ASBS #29) in La Jolla, CA according to requirements in the California Ocean Plan. Samples were collected during three storm events that occurred between November 2011 and March 2012. Additional monitoring was conducted during a March 2012 storm event at one of these outfall discharge locations as part of a State of California Proposition 84 funded program. This outfall location consists of a protected rocky intertidal habitat that experiences inundation with freshwater during storm events occurring at low tide. The additional monitoring included laboratory toxicity testing of receiving water from several sites within and surrounding the intertidal area; samples were collected prior to and at multiple intervals during a storm event. This comprehensive study also included *in situ* toxicity monitoring, collection of samples for chemical analyses, and storm water plume characterization, with the goal of determining potential impacts of storm water on the beneficial uses of the La Jolla coastal region. Effects on mysid shrimp (*Amercamysis bahia*) acute survival and purple urchin (*Strongylocentrotus purpuratus*) embryo-larval development were observed in several storm water samples collected as a part of this monitoring program. Purple urchin embryo-larval development was also inhibited to a lesser degree in multiple receiving water samples. Toxicity identification evaluations (TIEs) were conducted on samples that exhibited a substantial response. Adverse effects on purple urchin embryo-larval development observed as a result of exposure to storm water samples were attributed to metals, and reduction in mysid survival was mostly likely caused by synthetic pyrethroid pesticides. The effects on purple urchin embryos exposed to receiving water samples has proved much more difficult to attribute to a specific constituent, as observed responses are not persistent, and a high variability in response has been observed spatially, temporally, and among individual test replicates. Non-polar organic compounds have been ruled out as a contributor through TIE investigations, and common anthropogenic trace metals (i.e. copper and zinc) are an unlikely cause based on concentrations measured. Marine algal species are currently suspected and this hypothesis under investigation.

**WP102 Evaluation of tire and road wear particles in the Chesapeake Bay watershed: A sustainability initiative of the global tire industry** K.M. Unice, J. Chu, J.M. Panko, ChemRisk. The Tire Industry Project (TIP) is a sector project organized under the World Business Council for Sustainable Development that has been engaged in a multi-year project to understand the potential for environmental health risk associated with tire and road wear particles (TRWP). Therefore, a global sampling study was conducted to understand the potential for exposure to TRWP with the objective of quantifying tread levels in air and sediment at locations that were representative of potential human and ecological exposure. One of the three global study areas was the Chesapeake Bay watershed in the United States, which was selected to characterize a high population density area that also contained a range of rural and urban locations. Individual sampling sites were chosen to represent a diversity of human receptors and ecological habitats with proximity to identifiable vehicle traffic sources. Other factors of consideration included traffic load, population density, land use, availability of historical sampling data, and sample point accessibility. To quantify tread particles in air and sediment within the watershed, a chemical marker specific to the polymer portion of TRWP was used. The average tread particle PM10 concentration for the air samples ( $n = 27$ ) was  $0.068 \mu\text{g}/\text{m}^3$ , representing an average contribution of 0.83% to the overall PM10 in the watershed. Analysis of the sediment samples ( $n = 50$ ) resulted in an average tread concentration of 460 ppm (100% detection frequency). Air-borne tread concentrations varied with traffic load and population density; however, the trend was not statistically significant. For sediment, total organic content, sediment grain size, and population density were significant determinants of tread concentration. The tread marker results significantly correlated with a number of other traffic associated metals, such as cobalt and zinc, increasing confidence in the accuracy of the pyrolysis marker. The results of this study suggest that tread particles are reliably quantified by the polymer pyrolysis marker and that TRWP are widely dispersed in the environment.



**WP103 Exposure of fish in the Athabasca and Slave Rivers to PAHs potentially derived from Oilsands operations** E. Ohiozebau, University of Saskatchewan / School of Environment and Sustainability; B. Tendler, A. Hill, University of Saskatchewan; G. Codling, University of Saskatchewan / Toxicology Centre; J.P. Giesy, University of Saskatchewan / Biomedical Veterinary Sciences & Toxicology Centre, Michigan State University / Department of Animal Science, University of Saskatchewan / Toxicology Centre; E. Kelly, Government of the Northwest Territories / Environment and Natural Resources; P.V. Hodson, Queen's University / School of Environmental Studies; J. Short, DJWS Consulting LLC; P. Jones, University of Saskatchewan / School of Environment and Sustainability, University of Saskatchewan / School of Environment and Sustainability and Toxicology Center. A large proportion of the Alberta oilsand open-pit mining activities are near to or adjacent to the Athabasca River. Atmospheric emissions and oil sands process-affected waters (OSPW) contain a cocktail of chemicals including polycyclic aromatic hydrocarbons, (PAHs), naphthenic acids and metals, some of which are persistent, toxic, mutagenic and potentially carcinogenic, though there is still much we do not know about their environmental fate. Concerns have been expressed about the potential impacts to the environment and human health by communities in the Athabasca River basin as well as by communities in the downstream Slave and Mackenzie river basins. These concerns have been exacerbated by uncertainties concerning the origin of contaminants, as both metals and organic compounds may be from natural as well as anthropogenic sources. The spatial and temporal distribution of PAHs in fish bile, liver and muscle from selected locations along the Athabasca and Slave Rivers, were collected. Target sample size for the project was 30 individuals of 5 species (Goldeye – *Hiodon alosoides*, Whitefish – *Coregonus clupeaformis*, Northern Pike – *Esox lucius*, Walleye – *Sander vitreus*, Burbot – *Lota lota*) at three location on the Athabasca river and two locations on the Slave River. Fish were sampled in summer, fall and winter of 2011 and a total of 834 fish were sampled. Bile PAH profiles were determined by synchronous fluorescence spectroscopy followed by quantitative LC/MS/MS and GC/MS to determine individual parent and alkylated PAHs. Fish sampled from upstream Athabasca River locations close to operational sites have a greater exposure to PAHs than fish from the Slave River. Bile PAH profiles also suggest different sources of PAHs on the Slave and Athabasca rivers. At the Slave River sites lower molecular weight PAHs represent a greater proportion of the total bile PAHs compared to the Athabasca River sites.

**WP104 Extent of Hypoxia in the Southern California Bight Estuaries and Relationship to Environmental Drivers** K. McLaughlin, M. Sutula, Southern California Coastal Water Research Project / Biogeochemistry Department; J. Crooks, Tijuana River Estuary National Estuarine Research Reserve; D. Gibson, San Elijo Lagoon Conservancy; L. Busse, San Diego Regional Water Quality Control Board; S. Berquist, Santa Monica Bay Restoration Commission; R. Dagit, Resource Conservation District of the Santa Monica Mountains; S. Anderson, CSU Channel Islands / Biology department; L. Stratton, UC Santa Barbara. Eutrophication of estuaries and coastal waters is a global environmental issue, with demonstrated links between anthropogenic changes in watersheds, increased nutrient loading to coastal waters, harmful algal blooms, hypoxia, and other effects on aquatic food webs. In southern California, eutrophication in estuaries has not been well studied. The Bight 08 Estuarine Eutrophication Assessment sought to rectify this data gap by assessing the magnitude and extent of eutrophication and the linkage to nitrogen (N) and phosphorus (P) loads at 27 sites in 23 estuaries in the Southern California Bight (SCB) for the 2008-2009 water year. Indicators of eutrophication consisted of primary producer abundance (macroalgae and phytoplankton), measured bi-monthly, while dissolved oxygen (DO), chlorophyll a, and other physiochemical parameters that were monitored continuously in bottom waters in each site. Nitrogen (N) and phosphorus (P) loads were estimated through empirical measurements and modeling. Using the European Union Water Directive Framework, 63% of sites a large fraction of segments had moderate or lower ecological condition (78% based on macroalgae, 39% for phytoplankton, and for DO). Duration of hypoxia in Bight estuaries typically occurred over a diel cycle, with 98% of hypoxic events < 24 hours in duration. While primary producer abundance was significantly correlated to nutrient loads, extent of hypoxia had no significant correlation with loads; instead, we found that extent of hypoxia was significantly correlated with sediment organic carbon and total nitrogen, suggesting it integrates the effects of nutrient loading over longer time scales compared to primary producers.

**WP105 Findings of a Review of Science Supporting Dissolved Oxygen Objectives in California Estuaries** M. Sutula, Southern California Coastal Water Research Project / Biogeochemistry Department; H. Bailey, Nautilus Environmental; S. Poucher, Science Applications International Corporation; C. Swift, Enterix Inc.. California State Water Resources Control Board (SWRCB) is developing nutrient water quality objectives for the State's surface waters, using an approach known as the Nutrient Numeric Endpoint (NNE) framework. The NNE establishes a suite of numeric endpoints based on the ecological response of an aquatic waterbody to nutrient over-enrichment (eutrophication, e.g., dissolved oxygen, algal biomass, etc.). The SWRCB intends to use dissolved oxygen as one of the indicators in the NNE for estuaries. The purpose of this study was to evaluate the current scientific basis supporting derivation of objectives for dissolved oxygen for estuaries and enclosed bays in California, with the exclusion of the San Francisco Bay-Delta Estuary. The scientific approach used in this effort is adapted from the Virginia Province Salt Water Dissolved Oxygen Criteria, in which existing literature reviewed for fish and invertebrate indicator species or family "surrogates" to document tolerance to low dissolved oxygen. Two types of data were sought: 1) Acute data used to derive a maximum concentration (minimum concentration in the case of DO) to protect against short-term mortality, and 2) chronic data used to derive a value protective of lethal and sublethal effects under long-term exposure conditions. The DO tolerance data were then evaluated to establish their suitability for derivation of DO criteria for protecting organisms associated with California estuaries. This talk will summarize the findings of this study, identify data gaps, discuss recommendations.

**WP106 Fish health in the Reedy-Saluda watershed** P. van den Hurk, Clemson University / Biological Sciences; J. Mierzejewski, Clemson University / Department of Biological Sciences; D. Haney, Furman University / Biology. The water quality of the rivers in the SC Upstate has been of concern for years. Legacy pollutants that were produced and discharged over the last century have accumulated in the sediments of a variety of reservoirs. Relatively recent environmental disasters and continuous discharge of environmental pollutants through wastewater treatment plants and non-point sources keep putting strains on the quality of essential surface water resources. In 2009 the Saluda River was ranked as # 6 of America's Most Endangered Rivers by American Rivers. Water quality in the upstate rivers has been measured extensively over the last two decades by local and State platforms, which produced a number of papers evaluating the water quality condition of the watershed. However, very little has been contributed to effects that reduced water quality might have on fish health in the rivers that are part of the Saluda-Reedy watershed. We started investigating fish health through biomarker studies in the Reedy River reservoirs in 2004. In subsequent years the main stem of the Reedy River was sampled, followed by samples from urban creeks in the City of Greenville and an extensive sampling program in the Saluda River in 2010. The results of our studies show that a variety of sources appear to be responsible for the strain on the biochemical homeostasis of fish species in the Upstate rivers. Examples will be presented of effect-inducing pollutant sources like the polynuclear aromatic hydrocarbons (PAHs) in the sediments of Lake Conestee, estrogenic compounds in discharges from wastewater treatment plants, PAHs in run-off from parking lots and driveways, and the compounding effects of mixture exposures in urbanized areas. Future analyses will focus on the connection between biomarker responses in less sensitive fish species and the disappearance of more sensitive species from the species spectrum. The rivers in the Upstate of SC have an exceptional reputation of species richness and uniqueness; biomarker studies will help to monitor and protect this precious natural resource which is also essential for a variety of societal uses like recreation and drinking water supply.

**WP107 Four year study assessing effectiveness of BMPs in three upper subwatersheds of the Strawberry River, AR, Fulton Co, USA** T.R. Brueggan, Arkansas State University; J.L. Bouldin, Arkansas State University / Department of Biological Sciences, Arkansas State University, Environmental Sciences Graduate Program / Environmental Sciences Graduate Program, Arkansas State University / Department of Environmental Science. Agricultural activities continue to negatively influence surrounding waterways despite continual studies on the use of Best Management Practices (BMPs) to limit such impacts. A variety of BMPs studied are attempts to control soil erosion therefore limiting the harmful effects linked to excess sediment in waterways. These practices have been shown to be effective at preserving

natural water quality. This four year study is focused on six collection sites, three in the upper portion of BMP implementation and three below BMP implementation, located in the upper subwatersheds of the Strawberry River, AR. Multiple BMPs including the exclusion of cattle from waterways while providing alternative water sources and use of no-till method to plant pasture grasses are being implemented. This presentation will provide a summary of four years of data collection to assess the effectiveness these BMPs. Results from chemical testing include total suspended solids, chlorophyll-*a* and nutrients including ortho-phosphates, nitrates and nitrites. Results from biological testing include *Escherichia coli*, Whole Effluent Toxicity (WET) testing using *Ceriodaphnia dubia* and *Pimephales promelas* and sediment testing using *Chironomus dilutus*. Significant aqueous and sediment toxicity has occurred throughout the study. Results of all analyses indicate the necessity of long-term studies to accurately assess the effectiveness of BMPs as results vary both spatially and temporally.

**WP108 Health Status and Biomarker Responses in Fish from the Athabasca and Slave Rivers in Relation to Potential Exposure to Oilsands Contaminants**

**P. Jones**, University of Saskatchewan / School of Environment and Sustainability, University of Saskatchewan / School of Environment and Sustainability and Toxicology Center; E. Ohiozebau, University of Saskatchewan / School of Environment and Sustainability; B. Tendler, University of Saskatchewan / School of Environment and Sustainability and Toxicology Center; A. Hill, University of Saskatchewan / School of Environment and Sustainability; G. Codling, University of Saskatchewan / Toxicology Centre; J. Giesy, University of Saskatchewan / Veterinary Biomedical Sciences; E. Kelly, Government of the Northwest Territories / Environment and Natural Resources; P.V. Hodson, Queen's University / School of Environmental Studies; J. Short, DJWS Consulting LLC. A large segment of the Alberta oil sand open-pit mining activities are adjacent to the Athabasca River. Aerial emissions and oil sands process-affected waters contain many different chemicals including polycyclic aromatic hydrocarbons (PAHs), naphthenic acids, and metals, some of which are persistent, toxic, mutagenic and potentially carcinogenic. Concerns have been expressed by communities in the Athabasca River basins as well as by communities in the downstream Slave and Mackenzie river basins about the potential environmental and human health effects of emissions. These concerns have been exacerbated by uncertainties concerning the origin of these contaminants. The aim of this research was to describe the current status of fish health in the Athabasca and Slave rivers in relation to concentrations of metals and PAHs measured in fish tissues. Target sample size for the project was 30 individuals of 5 species (Goldeye – *Hiodon alosoides*, Whitefish – *Coregonus clupeaformis*, Northern Pike – *Esox lucius*, Walleye – *Sander vitreus*, Burbot – *Lota lota*) at three locations on the Athabasca river and two locations on the Slave River. Fish were sampled in summer, fall, and winter of 2011. A total of 834 fish were collected. At the time of collection all fish were subjected to a standardized and detailed health assessment including both external and internal examinations. Tissues were preserved for chemical analyses and for histological, biochemical, and molecular biomarkers. External and internal indices of fish health showed no significant differences related to proximity to oil sands operations or location on the river system. These results will be discussed with reference to the observed concentrations of PAHs and metals measured in the fish and the biochemical markers analysed. While chemical residue data indicates exposure of fish to contaminants, there appears at this time to be little or no impact on fish health in the Athabasca/Slave river system.

**WP109 Response of stream algal assemblages in an agricultural basin during a peak herbicide application period**

**T.B. Orr**, Monsanto Company / Cooperative Wildlife Research Laboratory, Monsanto Company / Ecotoxicology and Environmental Assessment; L.J. Schuler, Monsanto Company / School of Biological Sciences, Monsanto Company / Ecotoxicology and Env. Risk Assessment; J. Wright, Monsanto Company; J. Honegger, Monsanto Company / Ecotoxicology & Environmental Risk Assessment Team. Herbicide water quality standards and impairment criteria are based on laboratory derived toxicity values to individual algal species and do not take into account changes in algal communities, the typical resource to be protected. A study was conducted in June 2008 in the Le Sueur River watershed in south-central Minnesota to inventory algal communities during a period of peak flow when highest herbicide concentrations in surface water could be expected. The Le Sueur River watershed is comprised primarily of agricultural land uses with corn production constituting the primary land use. The

objectives of the study were to inventory and assess algal community composition in stream reaches of the Le Sueur River watershed and to gather information within the Le Sueur River watershed regarding factors that may influence algal community abundance, diversity and health, including information related to stream water quality and stream channel characteristics. The results of this study indicate that stream algal communities are highly variable and are influenced by a complex interaction of environmental factors. Of these factors, physical stressors such as current velocity during peak flow periods have the greatest influence on determining stream algal community composition. Future assessments of algal community impairment due to agricultural inputs should also account for varying flow regimes within a basin. Additional studies are warranted to more thoroughly investigate the relative contribution of physical versus agricultural stressors on the algal communities and to understand the time to subsequent recovery.

**WP110 Sediment and nutrient reduction initiatives in the Arkansas Delta Ecoregion**

**J.L. Bouldin**, Arkansas State University / Department of Biological Sciences, Arkansas State University, Environmental Sciences Graduate Program / Environmental Sciences Graduate Program, Arkansas State University / Department of Environmental Science; M.L. Reba, US-DA-ARS National Research Service; T.G. Teague, Arkansas State University. Federal initiatives such as the Mississippi River Basin Initiatives (MRBI) are aimed at reducing the hypoxic zone in the Gulf of Mexico. Direct tributaries of the Mississippi River are cited as having greater contribution to the hypoxic zone. As the Arkansas Delta Ecoregion is an extensively cropped agricultural region with direct drainage to the Mississippi River, it is a focus area for reduction in agricultural runoff. Row-crop agriculture is the primary land use in the Arkansas Delta and thus enhancing conservation practices that reduce sediment and nutrient loss from production fields will decrease loading to receiving streams. Select watersheds in the Delta are targeted for conservation implementation and continual water quality measures will detect reduction in non-point source contamination from agriculture. Both instream and edge-of-field sites are monitored as part of this study to compare efficacy of conservation practices as runoff leaves production fields and is subsequently transported to downstream receiving systems. Temporal spikes over the course of a runoff event were measured in nutrient and suspended sediment concentrations from edge-of-field samples. Seasonal differences were also measured in samples collected instream and edge-of-field. These two sampling practices will provide insight to better compare direct field runoff and water collected from receiving systems.

**WP111 Water Quality Monitoring in the Cache River Watershed,**

**Arkansas** **C.A. Rosado-Berrios**, Arkansas State University / Department of Environmental Sciences; J.L. Bouldin, Arkansas State University / Department of Biological Sciences, Arkansas State University, Environmental Sciences Graduate Program / Environmental Sciences Graduate Program, Arkansas State University / Department of Environmental Science. The Bayou De View – Cache River Watershed is part of the White River Basin in Eastern Arkansas. The uppermost section of the watershed is located in the southeastern part of Missouri and runs through the northeast part of Arkansas in a south-southwest direction through the ecoregion known as the Delta. The Cache River lower section is used for fish and wildlife propagation as well as water source for primary and secondary recreation and for domestic, agricultural and industrial supply. This watershed is included in the Mississippi River Basin Initiative (MRBI) to reduce sediment and nutrient loading to the Gulf of Mexico while the upper watershed does not meet aquatic life use criteria designated due to the presence of toxic metals, elevated chlorides levels and total dissolved solids. The watershed is monitored for a three-year period to evaluate the basin water quality before, during, and after the initiation of conservation practices. Seven sampling sites are located in the lower subwatersheds to monitor pH, turbidity, dissolved oxygen (DO), conductivity, temperature, total suspended solids (TSS), and nutrient concentrations. Further testing will include presence and concentrations of lead and principal phthalates including possible source investigations. This work presents the data obtained during the first year of monitoring as baseline. Among all sites observed results included mean detected levels from below detection limits (BDL) to 0.458mg/L, 0.026mg/L and 0.119mg/L for dissolved nitrate, nitrite and phosphate, respectively. Means for TSS ranged from 4.09mg/L to 191.26mg/L, turbidity values as high as 356NTU were measured and the lowest DO level detected was 1.4mg/L. Long-term water quality monitoring in this watershed will provide evidence of conservation practices within the watershed.

**WP112 Assessment of polybrominated diphenyl ethers and 2,2',4,4',5,5'-hexabromobiphenyl in bottom sediment of some effluent-receiving Rivers in Cape Town** O.S. Olatunji, Cape Peninsula University of Technology / Department of Chemistry; J.P. Odendaal, Cape Peninsula University of Technology / Department of Environmental and Occupational Studies; A.P. Daso, Cape Peninsula University of Technology / Department of Food and Agricultural Sciences. Until recently, studies reporting the environmental levels of polybrominated diphenyl ethers and polybrominated biphenyls in Africa are rarely found in the literature. Consequently, this study was aimed to assess the concentrations of commonly investigated PBDE congeners (BDE 28, 47, 100, 99, 154, 153, 183 and 209) as well as BB 153 in bottom sediment of two rivers receiving effluent discharges within the City of Cape Town. In this study, both GC- $\mu$ ECD and GC-TOF-MS techniques were employed for the routine analysis and the structural elucidation of the target compounds, respectively. The overall mean concentrations of the total PBDE ranged from 0.06 – 2.47, 0.07 – 0.58 and 0.10 – 0.78 ng/g for the upstream, point of discharge and downstream sampling points, respectively. These values were relatively higher in the Kuils River samples where the overall mean concentrations were found to be 0.22 – 1.52, 2.36 – 9.95 and 0.31 – 5.38 ng/g for the upstream, point of discharge and downstream sampling points, respectively. The average levels of BB 153, which were generally low, ranged from ND – 0.33, ND – 0.22 and 0.04 – 0.39 ng/g for the upstream, point of discharge and downstream sampling points, respectively. Similarly, the average levels of BB 153 in the Kuils River were in the range of ND – 0.18, ND – 1.01 and ND – 1.21 ng/g for the upstream, point of discharge and downstream sampling points, respectively. The statistical analyses performed on these data showed that majority of the PBDE congeners, except BDE 209 had strong positive correlation with the total organic carbon (TOC). Furthermore, the Pearson's correlation analyses conducted amongst the congeners showed that majority of the PBDE congeners as well as BB 153 had strong positive relationship with one another, which were statistically significant at both 0.01 and 0.05 (p-values), thus suggesting the possible similarity in the sources of these pollutants into the investigated rivers. This study further confirms the potential of wastewater treatment plants' discharges as important source of PBDEs in the receiving environment.

**WP113 Nutrient enrichment and hypoxia threat in urban surface waters** O.S. Olatunji, O.S. Fatoki, Cape Peninsula University of Technology / Department of Chemistry; B.O. Opeolu, Cape Peninsula University of Technology / Department of Environmental and Occupational Studies; B.J. Ximba, Cape Peninsula University of Technology / Department of Chemistry; O.Z. Ojekunle, K.N. Adeniyi, University of Agriculture Abeokuta / Environmental Management and Toxicology. Water samples pooled from nine urban streams/rivers traversing high density residential and commercial areas in the Abeokuta metropolis, Ogun State, Nigeria, were investigated in order to determine the effect of inorganic nutrients on oxygen distribution between 2008 – 2010, using standard methods. Results revealed that the metropolitan streams/rivers are anoxic and hypoxia threatened due to high nutrient load (NL) except in peri-urban streams/river which served as control. The pH and concentrations of Dissolved Organic Carbon (DOC), Total Nitrogen (TN) and  $\text{P-PO}_4^{3-}$  of the streams/rivers were 6.6 – 6.9, 24.8 – 155.4 mg/L, 3.82 – 15.46 mg/L and 1.35 – 5.71 mg/L, respectively, while dissolved oxygen (DO) ranged between 3.25 – 7.46 mg/L. Results also showed that the biochemical oxygen demand (BOD) and chemical oxygen demand (COD) values ranged between 180.81 – 200.62 mg/L, 250.27 – 312.83 mg/L, respectively. Analysis of variance showed significant differences between sampling sites as well as seasons (wet and dry) in terms of NL and oxygen levels in all the stream/river water samples investigated, with those pooled from residential locations having higher NL values during the dry season. Thus DO levels in almost all the urban streams/rivers were low, hence the need for regulatory intervention to ensure point of compliance necessary to protect inland freshwater from sewage inlets.

**WP114 Assessing Pesticide Effects Using an AQUATOX Aquatic Ecosystem Model with FOCUS Exposure Scenario** D.V. Preziosi, Integral Consulting Inc; R.A. Pastorok, Integral Consulting; D.A. Rudnick, Integral Consulting, Inc; Y. Bramley, Syngenta; E. Farrelly, Syngenta / Ecological Sciences; D. Huggett, Syngenta; P. Thorbek, Syngenta Ltd / Environmental Safety, Syngenta / Environmental Safety. Aquatic ecosystem modeling can complement empirical approaches to pesticide risk assessment by allowing efficient evaluation of indirect effects mediated through species food-web

interactions, testing of multiple exposure scenarios, and assessment of potential long-term effects and recovery times. In a companion presentation, we report on the development and calibration of an aquatic ecosystem model based in AQUATOX (updated v. 3.0) for evaluating the potential direct and indirect ecological effects of prosulfocarb on freshwater systems. We report here on the expansion and application of the calibrated model to: 1) assess potential community-level effects of prosulfocarb in a generalized stream characteristic of agricultural areas of northern Europe, 2) demonstrate dose-responsiveness of the AQUATOX stream model, and 3) estimate recovery times of the biological community and key species for a hypothetical high-exposure scenario (concentrations 25X a realistic worst case). The R1 Stream Scenario of FOCUS (2001) was used as the basis for the site characterization and parameterization of physical and chemical elements of the stream model. A fish species (three-spined stickleback) and a dragonfly species (common club-tail) were added to the previously calibrated model, which already had phytoplankton, periphyton, zooplankton, benthic and epiphytic macroinvertebrates, and macrophytes. Information on toxicity of prosulfocarb was obtained mainly from the Draft Assessment Report developed by the responsible Rapporteur Member State. An annual time series of exposure concentrations for prosulfocarb was developed from a TOXSWA fate model based on the R1 Stream Scenario (i.e., a realistic worst-case scenario). All model species (or groups) showed negligible differences between control and prosulfocarb-exposed cases based on the FOCUS R1 Stream Scenario. Dose-responsiveness of the model was demonstrated by simulating a 25X-exposure scenario. Overall, the behavior of the mesocosm-mimic and generalized stream models follows an expected pattern for aquatic systems, with model species responding to physical-chemical conditions and trophic interactions in ecologically realistic ways.

**WP116 Deterministic and probabilistic approaches to estimate internal concentrations of an insecticide under different feeding scenarios in small rodents** A. Bednarska, Syngenta / Jealott's Hill International Research Centre; P. Edwards, Syngenta; R. Sibly, Reading University; P. Thorbek, Syngenta. Traditionally, quantification of chemical toxicity is based on measurements of external exposure that determines whether an organism will be affected by a toxicant in the environment. However, it is an internal concentration which drives the effect. To improve our prediction of the relationship between the external and internal concentrations of pesticides, we need toxicokinetic (TK) models which translate an external concentration of toxicant, which may change over time, to an internal concentration at target site. Based on the ADME (absorption, distribution, metabolism and excretion) data routinely generated as part of the data package for pesticides, we developed a body burden model which was applied to a variety of exposure scenarios, including one tested in a standard laboratory test on rats. Maximum internal concentrations ( $CM_{\text{Max}}$ ) for different combination of single fixed values for absorption  $k_a$  and elimination  $k_e$  rate constants and feeding patterns were compared to estimate risk, as for compounds that are excreted rapidly acute effects are usually associated with peak concentrations. Irrespective of  $k_a$  and  $k_e$  combinations, higher  $CM_{\text{Max}}$  were reached when the  $LD_{50}$  was given as a bolus dose and lower when more realistic scenario was used (i.e.,  $LD_{50}$  eaten with an intake rate changing over a two hour period according to pattern of short feeding bouts with breaks of low/no feeding activity). However, the lower the  $k_a$  and  $k_e$  the closer the feeding maximum body burden came to the bolus dose maximum body burden ( $CM_{\text{MaxBolus}}$ ). Deterministic approaches have traditionally been used to complete the risk-assessment process. However, to incorporate the full range of exposure and quantify variability and uncertainty on risk we explored probabilistic approaches. To determine the probability of exceeding  $CM_{\text{MaxBolus}}$  for individual rats feeding an insecticide under field application conditions, we will also illustrate probabilistic model using Crystal Ball Software which permits Monte Carlo analysis for simulations of real-world scenarios involving various elements of uncertainty.

**WP117 Generalized modeling of the hypothalamic-pituitary-gonadal axis: Feedback signaling on steroid hormone production** M. Mayo, US Army ERDC; E.J. Perkins, US Army Engineering Research & Development / Environmental Laboratory, US Army ERDC / Environmental Processes & Effect Division, US Army Engineering Research & Development / Environmental Genomics and Genetics Team; K.H. Watanabe, Oregon Health & Science University / Division of Environmental and Biomolecular Systems. Animal reproduction is potentially affected by chemicals in the environment that disrupt production of steroid hormones, such as 17 $\beta$ -estradiol



and testosterone. Hormone signaling between the hypothalamus, pituitary, and gonads (HPG) is regulated by a complex biochemical network rich in regulatory loops that use chemical signals as regulators of critical processes. Together with biotransformation and elimination mechanisms, these regulatory loops help to establish a system capable of compensating for and adapting to low and moderate chemical exposures. Exactly how these regulatory loops contribute to this system-level behavior is not entirely understood. Mathematical modeling is well-suited to this task. Here we employed a recently proposed methodology, termed generalized modeling, which eliminates the need to specify mathematical representations for chemical reactions. Instead chemical fluxes and steady state concentrations are used to investigate the system dynamics, in order to better understand the conditions under which different states of the HPG axis and corresponding steroid hormone concentrations may exist. Specifically, in the model teleost fathead minnow (*Pimephales promelas*), we investigated the influence of a negative or positive feedback between pituitary regulation of luteinizing hormone and plasma estradiol and testosterone levels. The role of other regulatory loops in the HPG axis on endocrine function will also be discussed.

**WP118 Herbicides and endangered butterflies: Can conservation practices imperil endangered species?** J.D. Stark, Washington State University, Puyallup Research and Extension Center / Department of Entomology, Washington State University / Dept. of Entomology; C. Johnson, U.S. Fish and Wildlife Service / Environmental Contaminants Division; N. Hanson, University of Gothenburg / Department of Plant and Environmental Sciences, University of Gothenburg / Plant and Environmental Sciences, EnviroPlanning. Lange's Metalmark butterfly (*Apodemia mormo langei*) an endangered butterfly species, is in imminent danger of extinction due to loss of habitat in the Antioch Dunes National Wildlife Refuge of California. Habitat loss is a result of invasive weeds which compete with the food of this species, Naked Stem Buckwheat (*Eriogonum nudum*). Various mechanical methods of weed control have not been successful and herbicides have been used for weed control in the refuge. However, concerns about the potential effects of herbicides led to toxicological studies on a related surrogate species, Behr's Metalmark (*A. virgulti*). We evaluated the toxic effects of three commonly used herbicides, triclopyr, sethoxydim, and imazapyr by exposing first instar larvae and monitoring them through their life cycle. Exposure to these herbicides resulted in significant reductions (24-36% less than the control) in the number of individuals that made it to the adult stage. Deterministic and stochastic matrix population models were developed to determine the potential impact of these herbicides on long-term population viability. Modeling results showed that exposure to each of these herbicides would result in increased probabilities of extinction and time to extinction compared to control populations.

**WP119 How are toxic effects on individuals translated to the population level?** B. Martin, Helmholtz Centre for Environmental Research / Department of Ecological Modelling, Helmholtz Center for Environmental Research; V. Grimm, Helmholtz centre for environmental research – UFZ / Department of Ecological Modeling, Helmholtz centre for environmental research – UFZ / Department of Ecological Modeling. We have developed a generic implementation of the Dynamic Energy Budget (DEB) theory in an individual-based (IBM) context <http://cream-itn.eu/projects/wp-1/daphnia-2/deb-ibm>. The combination of DEB and IBMs is an especially useful tool for population-level risk assessment, as DEB theory has been used extensively for the analysis of the lethal and sub-lethal effects of toxicants on individuals. The process-based approach used in the DEB analysis of toxicity experiments differs from standard ecotoxicological analysis (LC50, ECx, etc.) in that it links the effect to the internal concentration of the chemical and takes into account the biological characteristic of the test individual used. This is of great advantage in a population context because it allows us to extrapolate the effect of the chemical to among different size classes and to differing environmental conditions and exposure scenarios. Implementing DEB in an individual-based context allows for the extrapolation of effects measured at the individual level to the population level. Our initial work with DEB-IBM with *D. magna* has shown that with a relatively simple model we can replicate population dynamics (abundance and size structure) under control conditions. Using the same model we were able to predict the effect of the toxicant 3,4 dichloroaniline at the population level from data available in standard OECD tests. The advantage of developing such a model, is that when well tested, we can use these models to understand how

varying exposure scenarios, or environmental conditions would influence the population-level response to a toxicant. Lastly, because DEB can be used to model all animal species, we believe DEB-IBM is of specially value for population-level risk assessment because the same general model can be used for different OECD test organisms.

**WP120 Increasing Realism in Population Models for Assessing Pesticide Risks to Pacific Salmon** R.A. Pastorok, Integral Consulting; A. Parks, D.V. Preziosi, Integral Consulting Inc; N.N. Poletika, Dow Agro Sciences LLC / Field Exposure and Effects Department; M.F. Leggett, CropLife America. The National Marine Fisheries Service (NMFS) recently issued Biological Opinions (BiOps) regarding the use of certain organophosphate and carbamate pesticides in response to EPA consultation, mandated under the Endangered Species Act (ESA). The NMFS concluded that current and future uses of the pesticides considered would jeopardize the continued existence of listed populations of Pacific salmon. The potential influence of chronic sublethal exposures to pesticides was evaluated with population models. Because the results of this analysis formed the cornerstone of the jeopardy determinations made, it is appropriate to look at the model inputs and assumptions that were part of the population models used. We found several unrealistic assumptions underlying the models, particularly those regarding density-independent population growth, a constant exposure concentration, and outdated toxicity dose-response relationships. To evaluate the effects of these assumptions, we modified the BiOps models and developed a density-dependent age-structured model for stream-type Chinook salmon that also incorporated a chemical "decay" model and updated toxicity values, using chlorpyrifos as an example. The model used by NMFS predicted a significant decrease in  $\lambda$  at chlorpyrifos concentrations as low as 0.5  $\mu\text{g/L}$  for growth-mediated effects. In contrast, we found no significant ( $p > 0.05$ ) effects of chlorpyrifos on four population endpoints (total population density, annual intrinsic rate of population growth [ $\lambda$ ], Age 1 density, and first-year survivorship) for an annual-application scenario starting with a plausible peak exposure concentration of 1  $\mu\text{g/L}$ . Even in an unrealistic high-exposure scenario (initial 10  $\mu\text{g/L}$  chlorpyrifos), effects were found only on temporal phasing of population fluctuations, not on overall population densities and other endpoints. We conclude the population models used in pesticide BiOps should incorporate more realism to provide a technically defensible basis for jeopardy determinations under ESA.

**WP121 Mechanistic effect models can tell us when simplified risk assessment procedures are not adequate** A. Palmqvist, Roskilde University / Environmental, Social and Spatial Change, Roskilde University / Dept. of Environmental, Social & Spatial Change; M. Meli, Roskilde University / Dept. of Environmental, Social and Spatial Change; V.E. Forbes, University of Nebraska Lincoln / School of Biological Sciences. For Environmental Risk Assessment (ERA) of chemicals several assumptions are made in order to simplify testing procedures. The general idea is that the application of assessment factors covers uncertainty related to using simplified and short-term testing procedures, as well as extrapolating across different levels of biological organization. But unless it is known to what degree simplification results in imprecise estimates of risk, it is not possible to know whether current procedures lead to over- or under-protection. One assumption in ERA is that chemical exposure is well represented by a homogeneous exposure scenario, and researchers go to great lengths to ensure that tested chemicals are in fact homogeneously distributed in the test medium. In reality chemicals have a patchy distribution in the environment, in particular when it comes to chemicals associated with highly heterogeneous compartments such as soils and sediments, where environmental variables vary not only on a temporal, but also a spatial scale. To explore how spatial heterogeneity in chemical contamination influences the impacts of toxicants on populations of soil and sediment-living invertebrates, we used two types of mechanistic effect models, i.e., a metapopulation model and an Individual-Based Model. This modeling exercise has given us insight into which individual-level traits are responsible for population-level effects under homogenous and heterogeneous exposure scenarios. The major drivers of effects on population dynamics so far identified are organisms' dispersal ability and avoidance behavior. In addition, the results show that dose-response patterns and elasticities of individual life-history traits are important in determining the degree to which actual risk would be under- or over-estimated by ignoring spatial heterogeneity in habitat contamination. In conclusion, our results clearly show that mechanistic effect models can help us identify where and

to what degree we may under- or overestimate risk by using the current simplified risk assessment procedures.

**WP122 Modelling of interactions between toxicants and food – a case study for diquat in the pond snail *Lymnaea stagnalis*** E.I. Zimmer, Vrije Universiteit / Department of Theoretical Biology; V. Ducrot, L. Lagadic, INRA / Equipe Ecotoxicologie et Qualité des Milieux Aquatiques; T. Jäger, Vrije Universiteit. Toxicokinetic-toxicodynamic (TKTD) models are essential in the interpretation of toxicity tests with time-varying exposure, and the only possibility to extrapolate between different exposure scenarios. For sub-lethal endpoints such as growth and reproduction, the only mechanism-based TKTD approaches for these endpoints are derived from the Dynamic Energy Budget (DEB) theory. DEB theory provides a framework that explains how the energy taken up from food is allocated to the major physiological functions at the individual level. Internal concentrations from a TK model can be linked to changes in DEB parameters. Since food can be considered explicitly in a DEB framework, effects of food quality or quantity and potential interactions with toxicants can be considered. Although effects of food level and food source on toxicity have been discussed at length in the literature, their impacts are hardly quantitatively assessed. Food limitation often leads to higher apparent sensitivity (e.g., focusing on the EC<sub>x</sub>), which is not necessarily connected to an increased intrinsic sensitivity (i.e., the relationship between the internal concentration and the value of a DEB parameter). The pond snail *L. stagnalis* has been proposed as standard test organism for OECD guidelines for aquatic organisms, and the laboratory conditions are currently under investigation for suitability for standardization. Recently, it has been found that the pond snail *Lymnaea stagnalis* is food limited in a part of the life-cycle under the proposed test conditions, with severe consequences for the interpretation of toxicity test results. In this study, we use a model based on DEB theory to explain the response of two genetically different populations of the pond snail to pulsed exposure to the herbicide diquat, at four different concentrations. The hormetic pattern that is observed in the sub-lethal endpoints can be captured by effects on food intake only. The two populations only differ slightly in the parameters that determine the food limitation, however, this small differences lead to large differences in life history. We discuss the implications of the interpretation of the direct effects of diquat in comparison to indirect effects through the food used in the experiments. Further, we discuss possible improvements of both experimental setup and data analysis to avoid misinterpretation of test results.

**WP123 Population-level toxicant sensitivity: stage-specific density dependence and demographic stochasticity in Cu-exposed flour beetles (*Tribolium*)** J. Moe, Norwegian Institute for Water Research (NIVA) / Climate and environmental modelling, Norwegian Institute for Water Research / Climate and environmental modelling, Norwegian Institute for Water Resea / Section for Freshwater Biodiversity, Norwegian Institute for Water Research (NIVA) / Section for Climate and Environmental Modelling; P. Kramarz, S. Zmudzki, P. Gibas, Jagiellonian University / Institute of Environmental Sciences; A.B. Kristoffersen, Norwegian Veterinary Institute; N.C. Stenseth, University of Oslo. Population models are needed for predicting toxicants effects on population-level endpoints such as long-term abundance and extinction risk from measured individual-level endpoints such as survival and fecundity. Demographic processes such as density dependence and demographic stochasticity (random differences in vital rates among individuals) can complicate prediction of population-level effects. Stage-structured populations are particularly complicated; the different life-history stages may show different sensitivity to toxicant exposure, but the most sensitive stage is not necessarily the most important endpoint for the overall population-level effect. In this study we have investigated the role of stage-specific toxicant effects and stochasticity in populations of the red flour beetle (*Tribolium castaneum*) exposed to a toxic concentration of copper (Cu). We developed a population model based on the so-called LPA model (Larva-Pupa-Adult), which was developed for *T. castaneum* and has been applied extensively in population ecology for two decades. Density-dependent cannibalism across stages is driving the characteristic population dynamics in this model. Demographic rates (development time, juvenile survival and reproductive rate) in Cu-exposed and control populations of *T. castaneum* were estimated from a multi-generation experiment. Each treatment included 20 replicated populations, from which we could obtain estimates on uncertainty in demographic rates. The aim of this modelling study was to analyse: (1) effects of Cu-exposure on population-level endpoints such

as long-term abundance and risk of (quasi-)extinction; (2) sensitivity of the population-level endpoints to toxicant effects in the different life-history stages; and (3) the role of demographic stochasticity for the population-level toxicant effects. Preliminary sensitivity analyses indicate that changes in reproduction were more important for the population than changes in larval or adult mortality.

**WP124 Reliability of surrogate species: Assessing the relative merits of sublethal vs. lethal effects using life history data and matrix models** J.E. Banks, University of Washington / Environmental Science; J.D. Stark, Washington State University, Puyallup Research and Extension Center / Department of Entomology, Washington State University / Dept. of Entomology; A.S. Ackleh, University of Louisiana / Department of Mathematics. Although criticism of the use of surrogate species has been growing in applied ecology, a quantitative exploration of the relationship between life history ecology and reliable species protection at the population level has received little attention. We present a matrix model-derived closed-form mathematical expression that can be used to determine when surrogate species population outcomes will reliably predict outcomes of target (listed) species. Using an inequality that pinpoints conditions under which surrogate species are in fact reliable predictors of the fate of listed/threatened species, we compare the relative merits of parameterizing our model with fecundity (sublethal) vs. survivorship (lethal) life history values. In particular, we use case studies from endangered fish as well as parasitoid wasp populations to illustrate the utility of incorporating sublethal effects into these types of models. Survivorship parameters alone are often sufficient to identify the prevailing trends in population increase/decrease, but underestimate risk at the population level.

**WP125 Stochastic vs deterministic models: What's the value added by including variability and uncertainty?** R. Erickson, Texas Tech University / Dept. of Environmental Toxicology, Texas Tech University / Department of Environmental Toxicology, Texas Tech University / graduate student; S.M. Weir, The institute of environmental and human health / The institute of environmental and human health, The institute of environmental and human health / Environmental Toxicology; S. Cox, Texas Tech University / Dept. of Environmental Toxicology; C.J. Salice, Texas Tech University / Environmental Toxicology, Texas Tech University / TIEHH, Texas Tech University / Department of Environmental Toxicology. Population models can add value to ecological risk assessment by offering several advantages over other more traditional approaches. These advantages include the integration of individual-level processes, comparison of toxicant effects across different life stages, assessment of risk under different exposure and effect scenarios, and exploration of complex ecological risks. Despite these strengths, population models are still infrequently used in ecotoxicology. When used within ecotoxicology, population models are almost always deterministic and do not account for natural variability and uncertainty. We compared deterministic and stochastic versions of the same model to illustrate the differences of stochastic models and to determine any advantages of incorporating stochasticity. This was accomplished by adapting a previously published deterministic salmon population model to include stochasticity. The model examined the population-level effects of pesticide exposure to different salmon life stages. We adapted the model by replacing point estimates of life history parameters with distributions to reflect the uncertainty in estimations and natural demographic variability. Overall, the major findings were similar between the stochastic and deterministic models and showed that populations returned to equilibrium after exposure. However, the stochastic model revealed a nontrivial risk of extinction that was not apparent from the deterministic model. Furthermore, while there was agreement in recovery time among model constructs, the tail end of the recovery time distributions was longer in the stochastic model pointing to the importance of variability and uncertainty in predicting recovery. These might have important management implications for populations facing extinction risks from other stressors such as habitat loss and over harvest. Hence, incorporating stochasticity can enrich population models and may provide added insight into the range of potential outcomes.

**WP126 Toxicokinetics of metals in invertebrates: new mechanistic model supported by experimental data** R. Laskowski, Jagiellonian University / Institute of Environmental Sciences; E. Pyza, Jagiellonian University / Institute of Zoology; K. Argasinski, A.J. Bednarska, M. Choczynski, R. Sliwiska, Jagiellonian University / Institute of Environmental Sciences.

Toxicokinetics of metals is usually described by the one-compartment first-order kinetic model (herein called the 'classic TK model'). However, the model is purely phenomenological and does not seem acceptable on theoretical grounds. In the classic TK model the accumulation and excretion rates are kept constant while they may be expected to change depending on instantaneous toxicant concentration in the gut. Indeed, some researchers pointed out on discrepancies between predictions of the classic TK model and actual toxicokinetic observations. Recently we postulated that the main mechanism of metal toxicokinetics, at least in terrestrial invertebrates, is connected with the toxic effect of metals on gut epithelial cells. The constructed mechanistic model assumes a dynamic rate of degeneration and replacement of epithelial cells with increasing metal concentration in food. The model shows that the equilibrium concentration of a toxicant in an organism is the net result of gut cell death and replacement rates. The theoretical model formulation was shown to emulate well a broad range of toxicokinetic patterns, including those impossible to explain with the classic TK model. However, no data were available earlier to show if metals can really cause that substantial damage to gut epithelial cells. Also, the cell replacement rate was unknown, which hampered efforts in further model development. In this study we show that the exposure of the ground beetle *Perostichus oblongopunctatus* to high metal concentration indeed induced fast substantial degeneration of the gut epithelium. First apoptotic cells in the epithelium were observed after only one day of exposure. Their number increased gradually in consecutive days of feeding with Zn contaminated food. The apoptotic cells appeared first in the outer layer of the epithelium (after 4 days) and later in the whole epithelium (20 days). After 28 days of intoxication with Zn, massive degeneration of the epithelium was observed with many apoptotic cells in the lumen of the midgut. The epithelium was not recovered in the day 40, i.e., 10 days after the termination of Zn exposure. In the day 40 the epithelium was thin and had irregular structure. The changes in the gut epithelium were accompanied by a very high increase of body metal concentration in some individuals in the very first days since intoxication, followed by a decrease, probably as a result of shedding the degenerated gut epithelial cells.

**WP127 Which potential pesticide effects are dangerous to bird populations? Assessment using an individual-based modeling framework.**

K.A. Kułakowska, University of Reading / School of Biological Sciences; T. Kulakowski, University of Reading / School of Biological Sciences; R. Sibly, University of Reading. It is difficult to link wildlife exposure to pesticides and effect in population risk assessment. We may estimate the realistic exposure for individuals and we are aware what concentrations of a toxicant are likely to produce lethal or sublethal effects for individuals. However, this knowledge does not fully address the protection goal aimed at in the ecological risk assessment – the wildlife populations. Mechanistic effect modelling is the method used here to extrapolate from the knowledge of individual exposure and effects to population level effects. We have designed and implemented spatially explicit individual-based models for woodpigeon and skylark populations in the SkyPig modelling framework. The key processes included are: managing energy budget, foraging, territorial and breeding behaviour and mortality for birds and basic farming activities and vegetation dynamics for landscape. The models have been tested against data collected during long-term studies for both woodpigeon and skylark in modelled landscapes in the UK. The key question addressed here is: what kind and timing of individual-level effects is harmful for the population while affecting the smallest percentage of birds? The next question naturally follows: Are there any realistic exposure patterns that can cause these effects? To answer these questions we have selected a number of possible individual-level effects including, but not limited to delays in reproduction, increased egg and nestling mortality, abandonment of broods by parents and daily breaks in foraging. These have been applied in our models in different times of the year and affecting different percentages of a population. The population-level endpoints have then been compared with not affected population. Comparing the sensitivities of the populations of skylarks and woodpigeons to timing and scale of individual-level effects indicates the importance of considering inter-species variations in ecology for assessing the risk pesticides pose to them.

**WP129 A bioassay directed fractionation approach to discover novel bioactive organobromines from marine sponges and algae**

D.M Saunders, University of Saskatchewan / Department of Toxicology; R. Mankidy, G. Codling, H. Ma, University of Saskatchewan; J. Giesy, University of

Saskatchewan / Department of Toxicology. Organobromine compounds (OBrs) are known to be either synthetic or natural and have been used in a number of industrial processes as additives or final products. One such synthetic class of compounds, brominated flame retardants (BFRs), is found in various trophic levels of the food chain and has emerged as a contaminant of concern for human and animal health. In addition to the synthetic compounds, there are at least 1500 naturally occurring brominated compounds that have been identified in marine and terrestrial organisms. Several of these novel compounds have been shown to have bioactive effects ranging from anti-bacterial, anti-viral, anti-inflammatory, anti-cancer, to having anti-oxidant properties. The purpose of this project is to identify novel organobromines found in marine organisms including sponges and algae, to optimize current extraction and bioassay-directed fractionation techniques, and to assign bioactive properties to these compounds. Samples were extracted from homogenized red algae, *Porphyra haitanensis*, and *Gracilaria lemaneiformis* using an accelerated solvent extraction system with DCM/Hexane (1:1) and hexane/MTBE (1:1). The crude organic extract was assayed for cytotoxicity and anti-proliferation properties in the Hek293T cell line. Preliminary results indicated cytotoxicity and a decrease in cell proliferation at 1x concentration of the crude organic extracts. Investigation of additional properties of this crude extract is underway. A bioassay directed fractionation approach will be employed for further isolation/purification of bioactive fractions. Relatively pure bioactive fractions will be used for structural elucidation and quantification using mass spectrometric instrumental analysis. A high resolution gas chromatography mass spectrometry (HRGC-MS) system will be used for structural analysis with volatile fractions, and liquid chromatography mass spectrometry (LC-MS/MS), and quadrupole time of flight mass spectrometry (Q-TOF-MS) will be used for non-volatile fractions.

**WP130 An alternative reconstituted water formulation that better represents natural water composition**

S.M Hoheisel, c/o U.S. EPA / Student Contract Services; R. Erickson, J. Hockett, D. Mount, U.S. EPA. In previous work, we proposed a new formulation for reconstituted water for use in aquatic toxicity testing, one that had a composition more like natural waters compared to many reconstituted waters currently in use. This previous proposal relied entirely on readily soluble salts, specifically  $\text{CaCl}_2$ ,  $\text{CaSO}_4$ ,  $\text{MgSO}_4$ ,  $\text{NaHCO}_3$ , and  $\text{KHCO}_3$ . Using readily soluble salts is a convenience in the preparation of reconstituted water, but it comes at a price; without using the poorly soluble  $\text{CaCO}_3$  or  $\text{MgCO}_3$ , it is not possible to fully match typical composition of North American surface waters. This is because the soluble salt approach relies on  $\text{NaHCO}_3$  and  $\text{KHCO}_3$  to supply alkalinity, which directly results in excess Na and K in the water, and has the secondary effect of necessitating excess Cl and  $\text{SO}_4$ . In our current work, we use  $\text{CO}_2$ -assisted dissolution of  $\text{CaCO}_3$  and  $\text{MgCO}_3$  to provide alkalinity, which simultaneously allows a restoration of Na, K, Cl and  $\text{SO}_4$  to concentrations typical of natural waters. We provide procedural details on the preparation of this new reconstituted water, along with comparisons of its composition to natural waters. We also evaluated the compatibility of these waters with common test organisms using chronic tests with *Ceriodaphnia dubia* and *Daphnia magna*, and short-term chronic tests with fathead minnows (*Pimephales promelas*), and 10-d tests using *Chironomus dilutus* and *Hyalella azteca*. Using reconstituted waters that are more representative of natural waters should improve assessment of chemicals whose toxicity is influenced by the ionic composition of the water (e.g., many cationic metals), and may also improve performance of some organisms found to be sensitive to the ionic composition of water (e.g., *Hyalella*).

**WP131 Assessing Calculated GHS Classifications for Acute Aquatic Toxicity of Mixtures Against 96-Hour Static Fathead Minnow Test Results**

N. Pechacek, L. Zavada, T. O'Brien, Ecolab. The Globally Harmonized System of Classification and Labeling of Chemicals (GHS) represents an international approach to standardize the classification and communication of hazards for materials. Industrial products, which may be regulated under GHS, include cleaning products used in food and beverage industries. Such products commonly are discharged as part of plant effluent, which may or may not experience treatment prior to reaching aquatic environments. For appropriate product stewardship, such products should be assessed and appropriately classified per GHS for aquatic toxicity. For this study, commercial products used in food and beverage industries were initially assigned default GHS classifications for acute aquatic toxicity based on the conventional calculation method (CCM) as prescribed by GHS. For those



products with suspect or problematic default CCM classifications, aquatic testing was an option to assess such classifications. Of the over 900 products initially assigned CCM GHS classifications, 17 were selected for testing. The test used to challenge these default CCM classifications was a 96-hour acute toxicity static assay conducted with fathead minnow (*Pimephales promelas*). Testing was conducted following methodologies detailed in the 2002 USEPA document EPA-821-R-02-012 *Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms*. From these assays, fish 96-hour LC50 values were estimated and compared to GHS classification criteria to assign a test-based classification. Of the 17 products evaluated, three had test-based classifications that aligned with default CCM-based classifications. For the remaining 14 products, 13 had test-based classifications which were lower than the CCM classifications. Only one product had a test-based classification higher than the default classification. Based on this particular dataset of 17 products, the results suggest the CCM is generally more conservative than one based on 96-hour static testing with fathead minnows. A larger dataset is needed to make a definitive conclusion for this observation. It is also interesting to note that for the 13 products with less conservative classifications based on testing relative to CCM, 6 decreased one GHS category, 2 decreased two categories and 5 decreased three categories. Understanding potential factors influencing these discrepancies is of interest and an area of future work.

**WP132 Changes of metabolic profiles in Japanese medaka (*Oryzias latipes*) to dissimilar or similar binary stressors** S. Uno, Kagoshima University / Faculty of Fisheries; K. Nakayama, Ehime University / Center for Marine Environmental Studies; E. Koshiki, Kagoshima University / Faculty of Fisheries; A. Shintoyo, Kagoshima University; J. Koyama, Kagoshima University / Faculty of Fisheries. In the real environment, aquatic organisms are received several stress such as changing temperature and water qualities, exposures of chemicals, and others. The mechanism of effects from multiple stressors generally complicate and this explore is difficult. In present study, we used metabolomics approach using GC/MS and analyzed metabolic profiles in Japanese medaka (*Oryzias latipes*) received 1) dissimilar stressors as fasting and cold shock, or 2) similar stressors as exposure of phenanthrene and pyrene. The metabolites with significantly different levels among treatment group with either or both stress were screened by one-way analysis of variance (ANOVA). Principal component analysis (PCA) was then applied to the screened metabolites data, and principal component (PC) scores for each sample were obtained. In the exposure of dissimilar exposure, the group exposed to both stressors was clustered away from the groups exposed to either fasting or cold shock on two dimensional PCA score plot and almost placed at the sum of two vectors for groups individually exposed to fasting or cold shock. Especially, the metabolic effects to fasting were serious. For metabolites in TCA cycle, citrate and 2-oxoglutarate increased, although others as succinate, malate, and fumarate decreased. This disturbing could induced the shortage of energy, because 3-hydroxy butyrate dramatically increased and that increase indicates to cause  $\beta$ -oxidation with shortage of energy only from acetyl-CoA. In the exposure of similar exposure, each individual exposure group of phenanthrene and pyrene clustered at similar place on PCA score plot. This result suggests that the effects to individual exposure of phenanthrene and pyrene were similar in metabolite profiles of medaka. On the other hand, the group exposed to both of phenanthrene and pyrene was clustered away from individual exposures. This suggests that the combined exposure of both PAHs induced some different effects to medaka metabolites from those of individual exposure. Only the combined exposure group was affected to the metabolism related to amino acids, and this phenomenon was not observed in individual exposure groups.

**WP133 Compilation and Evaluation of Associations between Deformities, Erosions, Lesions and Tumors (DELT Anomalies) in Fish and Stressors for EPA's CADDIS** J. Burris, CDM Smith; G.W. Suter, U.S. Environmental Protection Agency / National Center for Environmental Assessment, U.S. EPA / National Center for Environmental A; J. Gerritsen, Tetra Tech Inc.. Data were compiled and evaluated on associations between external deformities, fin erosions, lesions and tumors (DELT anomalies) and their physical, chemical and pathogenic causes. The goal was to identify causes associated with specific anomalies, the levels of exposure associated with those causes and the scientific quality of the information supporting the causal relationship. A literature search was initiated that included the development of a literature search strategy, completion of the search (both electronic and manual), review of the results using citation management

systems (Endnote and ProCite), and identification of relevant literature. Data from the relevant literature were extracted and coded into a database according to established coding guidelines that recorded the specific information for each association. Each association was scored for quality and strength of the association between cause and anomaly. The results were tabulated to show stressor levels (also chemical concentrations) associated with specific DELT anomalies. This presentation will review the results of the evaluation of the associations and will present the trends in the results and predominant causes of deformities, erosions, lesions and tumors in freshwater fish. Common causes include chemical, physical and pathogenic stressors. The strength of associations will be key to the discussions.

**WP134 Development and validation of the KOREA Insecticide Exposure Model (KOIEM) for Managing Insecticides** Y. Lee, Korea Institute of Toxicology / Future Environmental Research Center; Y. Kim, E&WIS Inc. / CEO; S. Lee, Korea Institute of Toxicology / Future Environmental Research Center, Korea Institute of Toxicology / Ecotoxicology Laboratory. The Korea Insecticide Exposure Model (KOIEM), multimedia fate model for estimating insecticide concentrations, was developed to facilitate ecological risk-based management of Korean insecticides. KOIEM simulates the fate and transport of insecticides sprayed in air and transported into water through soil and vegetation. We investigated application sites of insecticides all over the country (i.e. Korea), and standardized spatial scope of Korean water types. Based on sensitivity analysis, spray method, spray amount were very sensitive parameters to affect predicted environmental concentrations in surface water. Monitoring study was carried out on three water types (Stream, Ditch and Pond) in Jinju and Daejeon for two insecticides. Accuracy Factor (AF) as the ratio of Predicted Environmental Concentration (PEC) to Measured Environmental Concentration (MEC) was less than 10. We assessed ecological risk of several popular insecticides used in Korea.

**WP135 Development of Water Quality and Sediment Criteria in the Absence of Promulgated Standards; A Case Study with Aromatic Amines** E. Dillon, CH2M Hill; C.J. McCarthy, CH2M Hill / Environmental Services. Responsible parties often are often required to delineate chemicals beyond the boundaries of their properties. When the chemicals exceed MCLs or other applicable drinking water standards and have the potential for reaching waterbodies then they must also be delineated within surface water and sediment s of receiving waterbodies. The list of chemicals with drinking water standards is more extensive than the list of chemicals with promulgated surface water or sediment benchmarks. Responsible parties can be left cleaning surface water up to drinking standards or developing alternate benchmarks more appropriate for assessing potential risks to aquatic species. Using aromatic amines as an example, various methods for developing criteria were explored including: Quantitative Structure Activity Relationship (QSAR), Ecological Structure Activity Relationships (ECOSAR), and equilibrium partitioning (EQP). Challenges in the application of these approaches are presented. Some of the decisions in the process include: How much data is sufficient? Can freshwater species be used for marine environments or vice versa? Should a surrogate or model be used when insufficient data are available for a chemical? In many cases, there is no guidance or regulation driving these decisions leaving the responsible party to negotiate the outcome. The results of this work show that broad differences in final criteria can result depending on the answers to these questions.

**WP136 Diet and social facilitation impact life history and toxicant tolerance in freshwater snails: implications for laboratory studies** M.A. Funkhouser, Texas Tech University / TIEHH, Texas Tech University; S. Plautz, Texas Tech University / The institute of environmental and human health, Texas Tech University; C.J. Salice, Texas Tech University / Environmental Toxicology, Texas Tech University / TIEHH, Texas Tech University / Department of Environmental Toxicology. A primary goal of ecotoxicological studies is to generate data for use in environmental assessments. The underlying assumption is that toxicant-induced effects observed in the lab represent what may occur in the field. Although not well studied, laboratory conditions such as diet may have strong impacts on laboratory animals and the results of toxicity tests. *Biomphalaria glabrata* is a hermaphroditic, freshwater pulmonate snail commonly used to study host-parasite dynamics, adaptation to stressors, genetics, and toxicant sensitivity. We measured the effect of diet and social facilitation on snail reproduction, growth, and developmental toxicity. Beginning at 6 days old, snails from laboratory cultures were provided one of four common laboratory diets for 13

weeks: uncooked romaine lettuce (UL), fish food (FF), cooked romaine lettuce (CL), and cooked romaine lettuce plus fish food (CLFF). Within each diet, we evaluated the impact of social facilitation by rearing snails individually or in groups of three. To determine the impact of parental diet on developmental sensitivity to stressors, egg masses from study snails were subjected to 8ppm malathion or 25ppb cadmium. Diet significantly affected growth and reproduction. For example, CLFF snails had greater growth and reproductive success compared to snails on the other diets. There was also a significant effect of parental diet on sensitivity of developing embryos to cadmium. Eggs from parents fed UL had higher hatching success and lower time to hatch, but no difference was observed for malathion. Facilitation had a significant impact on reproduction; snails housed singly (self-fertilizers) produced more egg masses per snail per day, but less eggs per egg mass, than snails housed with conspecifics (outcrossers). Outcrossed snails produced egg masses that hatched more quickly and with greater hatching success compared to snails that reproduced via self-fertilization. As well, outcrossed snails produced eggs that were more cadmium tolerant than eggs from self-fertilizing snails. These findings suggest *B. glabrata* display the greatest reproduction and growth when fed CLFF and housed with conspecifics but displayed the greatest cadmium tolerance when reared on UL. The results have important implications for the study design of future laboratory experiments, both in *B. glabrata* and other snail species, particularly those capable of self-fertilization.

**WP137 Diet, predators, and partners: parental environments and experience impact the response of offspring to environmental stressors** S. Plautz, Texas Tech University / The Institute of environmental and human health, Texas Tech University; C.J. Salice, Texas Tech University / Environmental Toxicology, Texas Tech University / TIEHH, Texas Tech University / Department of Environmental Toxicology. Parental effects are nongenetic influences on offspring phenotype that result from the phenotype or environment experienced by the parents. Parental effects have been shown to be extremely important determinants of offspring fitness and have powerful implications for adaptive responses to stress. While parental effects are well studied in ecology and human health toxicology, relatively little is known about them in an ecotoxicological context, particularly with regard to our understanding of how populations respond to contaminant-induced stress. To better understand the role that parental effects have on the subsequent toxicant tolerance of their offspring, we exposed the offspring of two parental groups of the freshwater pulmonate snail *Biomphalaria glabrata* to cadmium and malathion challenges. Parental snails were raised in either a diet regime, in which they were fed uncooked lettuce, fish food, cooked lettuce, or cooked lettuce plus fish food, or a predator cue regime, in which they were exposed to a crushed snail plus crayfish water cue or control water. Within the diet regime, half the snails were housed singly and half in groups of three snails per container. *B. glabrata* is a self-fertilizing hermaphrodite that prefers to outcross so this last treatment was designed to evaluate the impacts of reproductive mode (self- or cross-fertilization) on offspring tolerance. In toxicant challenges, survival was recorded for cadmium every 24 hours for 4 days and for malathion at 3 and 24 hours post-exposure. The offspring of snails exposed to predator cues displayed greater cadmium tolerance ( $P \leq 0.001$ ), but malathion tolerance was not impacted by parental predator cue exposure at 3, 24, or 48 hours post-exposure ( $P \geq 0.05$ ). Cadmium tolerance varied with parental diet ( $P = 0.0423$ ) and was greater in the offspring of snails housed with conspecifics than alone ( $P \leq 0.001$ ). Malathion tolerance was not impacted by parental diet or housing with conspecifics at 3, 24, or 48 hours post-exposure ( $P \geq 0.05$ ). These results indicate that stressor tolerance in offspring can be heavily influenced by parental experience. Further, the results have important implications for the response of wild populations to anthropogenic stressors, particularly in complex, multi-stressor environment.

**WP138 Dietary antioxidants modulate the cellular and developmental toxicity of BDE 47** A. Yeh, C. Williams, University of Washington / Department of Environmental and Occupational Health Sciences; E.P. Gallagher, Department of Environmental and Occupational Health Sciences, University of Washington / Department of Environmental and Occupational Health Sciences. BDE 47 is a developmental toxicant and the predominant polybrominated diphenyl ether congener typically detected in fish tissues, including food species such as salmon. This observation has raised concern regarding the safety of salmon consumption. However, consumption of salmon is more routinely associated with protective health

benefits from antioxidant micronutrients, including omega-3 polyunsaturated fatty acids (OM3s). In the current study, we investigated the effects of dietary antioxidants, including OM3s relevant to salmon consumption, on the cellular toxicity of BDE 47. The zebrafish model was used to determine if antioxidants could protect against the *in vivo* developmental toxicity of BDE 47. Relative to control cells, HepG2 cells treated with an oxidized mixture of the two major salmon omega-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), showed increased viability and induction of antioxidant responses as evidenced by increased mRNA expression of glutamate cysteine ligase catalytic subunit (*GCLC*), and intracellular glutathione (GSH). Similar effects were observed in cells that were exposed to the model dietary antioxidant sulforaphane (SFN). Pretreatment with the salmon OM3s or with SFN protected against the BDE 47-induced loss of mitochondrial potential, but only marginally protected against BDE 47 cell toxicity. Similarly, SFN co-exposure provided partial protection against BDE 47 developmental toxicity in zebrafish, whereas co-exposure of embryonic zebrafish to N-acetylcysteine (NAC) significantly reduced the incidence of certain BDE 47 developmental defects, including atrioventricular blocks and tail curvatures. The results of these studies suggest an interaction between salmon OM3s and BDE 47 in human cells, including a partial protective effect of the antioxidants. Furthermore, our results indicate a role of oxidative stress in the *in vivo* developmental toxicity of BDE 47.

**WP139 Does holding back juvenile trout growth using restricted rations affect copper toxicity tolerance?** L. Beyger, University of Ontario Institute of Technology; J. Guchardi, University of Ontario Institute of Technology / Faculty of Science, Aquatic Toxicology, University of Ontario Institute of Technology / Senior Research Laboratory Technician; D. Holdway, University of Ontario Institute of Technology / Faculty of Science, Aquatic Toxicology, University of Ontario Inst. of Tech / Faculty of Science. Toxicity testing is a common method of practice when trying to establish guidelines and regulations for acceptable limits of chemicals being received in the environment. The aim of this research was to determine if some of the variability in  $LC_{50}$  values found in the literature for the same toxicants is due to researchers and/or hatcheries maintaining (zero growth) fish for extended periods of time prior to exposure. A standard 24-h flow-through  $LC_{50}$  of 44.5  $\mu\text{g/L}$  ( $\pm 3.54$ ) was determined for larval rainbow trout (~1.8 g wet weight) continuously exposed to copper. Another 24-h continuous flow-through  $LC_{50}$  of 50.4  $\mu\text{g/L}$  ( $\pm 3.98$ ) was determined after 21-d of holding at restricted rations (0.4 % body weight /day), the rainbow trout were approximately 2.1 g wet weight. There was no significant difference seen between these two  $LC_{50}$  values. Following another 21-d of holding at restricted rations, fish were ~2.8 g and a 24-h continuous flow-through  $LC_{50}$  of 66.5  $\mu\text{g/L}$  ( $\pm 1.41$ ) was calculated. This final  $LC_{50}$  value was significantly different ( $p \leq 0.05$ ) compared to the first and second values. The significant difference in the final  $LC_{50}$  value was likely due to the modest growth of the juvenile trout. Based on these findings, it is highly unlikely that the variability in  $LC_{50}$  values in the literature is due to the holding history of stock populations, including the use of restricted rations.

**WP140 Effective Strategies for Residual Polymer and Aquatic Toxicity Testing For Dredge Slurry Dewatering** B.A. Rabe, Environmental Resources Management, Inc., Environmental Resources Management; R. Wilcox, G. Lebster, WaterSolve, LLC. Chemical conditioning is typically required to facilitate the solids and water separation in the vast majority of dredging and dewatering operations. Even with the best efforts in optimizing the chemical dose, a small fraction of the polymer may be released in the filtrate water after the dewatering operations. Site conditions and regulatory requirements specific to a particular location may dictate that aquatic toxicity testing of the discharge water be completed on individual projects. After several products and/or combinations of products are tested in bench scale jar tests to determine the most effective products and the associated dose(s), a larger scale dewatering test is completed to determine or verify the suitability of the dewatering technology selected. The example projects discussed for this presentation utilized geotextile containers and the tests completed accordingly. The geotextile container filtrate collected from the laboratory scale tests was subjected in a series of aquatic toxicity tests based on regulatory agency requirements. Protocols for aquatic toxicity tests typically require both acute and chronic toxicity test assessments on a representative sample of filtrate water after conditioned with a specified chemical. In some cases, a specific dilution with site water will be included with the procedure to simulate a mixing zone. The species selected for testing will be



based on location, salinity, and other factors. It may be necessary to perform the aquatic toxicity testing using more than one chemical conditioning program. The regulatory agency will typically approve the chemicals at the dose used in the testing. Additional toxicity testing may be required during operations. Some projects have residual polymer testing requirements. Qualitative testing is typically done in the field as a gross determination of polymer present in the water. The quantitative test is a more precise test meant to be conducted in a laboratory setting with controlled conditions.

**WP141 Effects of Hardness and Alkalinity on the Growth of the Green Algae, *Pseudokirchneriella subcapitata*** D. Renfrew, Weston Solutions Inc / Natural Resources; A. Margolis, Weston Solutions, Inc.; B.J. Mastin, Weston Solutions, Inc. / Natural Resources. Municipalities are required to conduct urban runoff monitoring to comply with National Pollution Discharge Elimination System (NPDES) Permits. Often, these permits require the collection of samples to be tested for whole effluent toxicity that include an evaluation of growth inhibition using the green algae *Pseudokirchneriella subcapitata*, a species widely used to evaluate the potential for aquatic toxicity. Water samples collected in Southern California streams often exhibit higher hardness and alkalinity concentrations in comparison to the moderately hard control water used to identify relative inhibition. Existing permits and USEPA methodologies do not provide adequate guidance on how to handle control adjustments for high hardness and alkalinity site waters. Inhibition of *P. subcapitata* growth is frequently observed with samples collected in comparison to controls in the absence of toxic pollutants such as organics, herbicides, or metals. Toxicity Identification Evaluations (TIEs) conducted using C-18 filtration, ethylenediaminetetraacetic acid (EDTA), and sodium thiosulfate treatments did not affect toxicity. By comparing algae growth in these samples to a hardness and alkalinity control adjusted sample, growth was comparable between the two samples and suggested growth was not inhibited by a toxic constituent(s). A series of test solutions were then prepared to evaluate the growth effects of hardness and alkalinity concentrations ranging from 10 to 1,200 mg CaCO<sub>3</sub>/L hardness and 10 to 490 mg CaCO<sub>3</sub>/L alkalinity, respectively. Results and confounding factors are discussed which suggest the need for further guidance in future permits on how to evaluate results from high hardness and high alkalinity site waters.

**WP142 Effects of Ibuprofen on Rainbow Trout (*Oncorhynchus mykiss*) Following Acute and Chronic Waterborne Exposures** M. Robichaud, L. Beyger, University of Ontario Institute of Technology; R. Orrego, University of Ontario Institute of Technology / Faculty of Science, University of Ontario Institute of Technology / Faculty of Science, Aquatic Toxicology, University of Ontario Institute of Technology / post doctoral fellow; J. Guchardi, University of Ontario Institute of Technology / Faculty of Science, Aquatic Toxicology, University of Ontario Institute of Technology / Senior Research Laboratory Technician; D. Holdway, University of Ontario Institute of Technology / Faculty of Science, Aquatic Toxicology, University of Ontario Inst. of Tech / Faculty of Science. Pharmaceuticals and personal care products are a growing concern in the aquatic environment. Compounds from the class of non-steroidal anti-inflammatory drugs are commonly detected in surface waters and have the potential to negatively affect aquatic organisms. The purpose of this experiment was to evaluate the effects of ibuprofen on rainbow trout (*Oncorhynchus mykiss*) following acute and chronic exposures. Using waterborne exposures of trout to 1 and 10 mg/L of ibuprofen in the acute exposure and 1, 32 and 1000 µg/L of ibuprofen in the chronic exposure, along with an experimental control, E2 control of 1000 µg/L and an E2-ibuprofen mixed treatment, COX activity, vitellogenin (VTG) concentration and ethoxyresorufin-O-deethylase (EROD) activity were evaluated. COX activity measured showed that ibuprofen did not inhibit the enzyme activity in gill or kidney tissue. To evaluate the estrogenic effects of ibuprofen, VTG concentrations were measured and it was found that by the end of the chronic exposure all of the ibuprofen treatments had significantly increased VTG concentrations compared to the control group. EROD activity appeared to be inhibited by ibuprofen but definitive conclusions could not be made. These findings indicate that more work needs to be done studying ibuprofen in aquatic systems.

**WP143 Efficiency of chlorine toxicity removal of sodium thiosulfate and temporal changes in sensitivity of *Vibrio fischeri* in an Online Toxicity Monitor** J. Allen, U.S. EPA / Office of Research and Development, U.S. EPA / ORD; N. Muhammad, S. Panguluri, Shaw E&I. Aquatic Online

Toxicity Monitors have been developed to provide continuous, time-relevant information regarding water quality. These systems measure a physiological or behavioral response of a sentinel organism to water quality changes. One such system, the MicroTol Toxcontrol®, is based on the *Vibrio fischeri* model. Changes in luminescence of these bacteria over the course of a brief exposure period are an indication of the influence of water quality on the overall well-being of the organisms. This system is an autonomous, discrete exposure system which is intended to operate for a period of seven days with the same batch of bacteria in both source and drinking water distribution systems. Research presented here was designed to look at the ability of sodium thiosulfate to reduce chlorine toxicity and assess the sensitivity of *V. fischeri* over time. The *V. fischeri* assay requires an isotonic diluent prepared with 2% sodium chloride. An addition of 3ppm sodium thiosulfate was added to remove chlorine in chlorinated drinking water applications. Luminescence of control and treatment bacteria were measured simultaneously and used to compute percent inhibition. Two experiments are presented. The first is a series of 24 hour exposures comparing control water to additions of 3ppm sodium thiosulfate, 3ppm sodium thiosulfate and 1ppm Chlorine residual, and 3ppm sodium thiosulfate and 3ppm Chlorine residual. Results demonstrated that responses to control water and 3ppm sodium thiosulfate were equivalent and consistent over the 24 hour period. Exposure to 1ppm chlorine residual resulted in increased variation and positive response relative to the control. Variability was linearly related to the actual chlorine residual. Exposure to 3ppm chlorine residual resulted in a consistent negative response as was expected. The second experiment was a seven day test with daily exposure events to 10ppb copper. Results indicate that luminescence decreased over the seven day period in an exponential manner. Sensitivity of the bacteria to copper increased over the duration of the experiment following the pattern of initial luminescence decrease suggesting a relationship between sensitivity and batch age.

**WP144 Introducing Toxicity Testing to Mid- to High School Level Students: A Fun Teaching Activity Using *Daphnia*** A.A. Grippo, Arkansas State University / Department of Biological Sciences, Arkansas State University / Environmental Sciences Graduate Program; J.T. Kennon, Arkansas State University / Dept of Chemistry & Physics; J.M. Hall, Arkansas State University / Department of Mathematics and Statistics; K.L. Yanowitz, Arkansas State University / Dept of Psychology & Counseling; J.L. Bouldin, Arkansas State University / Department of Biological Sciences, Arkansas State University, Environmental Sciences Graduate Program / Environmental Sciences Graduate Program, Arkansas State University / Department of Environmental Science. As part of the Arkansas State University (ASU)/ NSF Noyce Scholarship program, we present an annual Try out the Classroom (TOTC) workshop to attract ASU science, technology, engineering & math (STEM) majors and graduates to science/math teaching. Funded by the NSF, the Noyce Scholarship program provides full financial support to upper level and graduate STEM majors to complete their BS/MS degrees; Scholars must commit to teaching science or math in underserved schools. Recently, TOTC participants designed and tested a simple, hands-on experiment that mimics real-life toxicity tests. This activity was developed as outreach for the ASU Ecotoxicology Research Facility (ERF), to be presented to mid- to high school level students who visit campus. The seven Noyce TOTC participants included undergraduates majoring in biology, agriculture, and chemistry, as well as two participants who had graduated from computer science and engineering programs. After touring the ERF, participants obtained *Daphnia* and moderately hard water used in toxicity tests, and chose NaCl as the "toxicant." After testing experimental variables (toxicant concentrations, time to toxic effect, number of organisms needed per sample, etc.), participants found that 3-5 *Daphnia* introduced to cups containing control water and five NaCl concentrations (0.1 – 0.5 g/50 mL) yielded a good viability curve within 30 mins. Food dye was used to further demonstrate serial dilutions; mathematics was introduced by calculating % live organisms, graphing results, and determining LD50. Participants researched background information on *Daphnia*, environmental toxicants, and toxicity testing, as well as AR frameworks for biology, chemistry and mathematics, and wrote a 6E lesson plan to be distributed to teachers. This activity has been useful as ASU ERF outreach; instructive to area students and teachers, introducing them to toxicity testing and increasing their awareness of environmental issues; and helpful to workshop participants in correctly designing a controlled experiment, as well as positively influencing their interest in teaching in a STEM field.



**WP146 Methods for culturing *Hexagenia* sp. and their use in bioaccumulation and toxicity testing** A.D. Harwood, Southern Illinois University / Fisheries and Illinois Aquaculture Center and Department of Zoology; A.K. Rothert, Southern Illinois University; M.C. Archer, Southern Illinois University / Chemistry and Biochemistry; M. Lydy, Fisheries and Illinois Aquaculture Center and Department of Zoology. The majority of sediment toxicity assessments are conducted using standardized species such as *Hyalella*, *Chironomus*, and *Lumbriculus*. However, the ecological relevance of these species, due to the relative sensitivity to native species is often questioned. The burrowing mayfly, *Hexagenia*, may serve as an ideal organism for both toxicity and bioaccumulation studies for several reasons. *Hexagenia* are recognized as sensitive species, have a long aquatic life stage (up to two years), and have large bodies, which aids in retrieval from sediments and for residue analysis. The current study outlines the Southern Illinois University – Carbondale established methods for collecting and hatching eggs, raising nymphs, and using these animals for bioaccumulation and toxicity testing. Furthermore, the relative sensitivity of three size classes to a representative contaminant (bifenthrin) was examined and compared to laboratory-derived and literature LC50s for the traditionally used *Hyalella* and *Chironomus*. Additional sublethal endpoints, including immobilization and suspended solids were defined for this species. This provides necessary guidance for the use of this species in toxicological bioassays.

**WP147 Reconstructing the past nutrient status of a Canadian Great Plains reservoir using depositional sediments and paleolimnological techniques** B. Lucas, K. Liber, University of Saskatchewan / Toxicology Centre; P.D. Jones, J.P. Giesy, H.S. Wheeler, L.E. Doig, University of Saskatchewan. Lake Diefenbaker, a reservoir created in 1967 in Saskatchewan, Canada, supplies drinking water to 45% of the province's population. Recent anecdotal evidence suggests that the frequency of cyanobacterial blooms is increasing within this reservoir. Toxins released from these blooms are a potential threat to the health of humans, livestock, and aquatic animals. Cyanobacterial blooms are known to emerge due to increased nutrient availability (typically phosphorus) and thus there is a need to investigate nutrient loading trends within the reservoir. Because limited empirical data are available to determine environmental trends, a paleolimnological study is being conducted to reconstruct the historical nutrient status of Lake Diefenbaker using physicochemical variables and subfossil remains archived in the sediment. The general objective is to determine if the nutrient status of Lake Diefenbaker has changed over time and whether this change is of concern. Cores of sediments were collected from deep-water locations near potential point sources of nutrients, and at corresponding reference sites. Cores were vertically sectioned into 1-cm increments and each layer analyzed for total phosphorus and three sedimentary forms of phosphorus (apatite inorganic, non-apatite inorganic, and organic phosphorus) to determine trends in phosphorus loading over time. Total sediment metals were determined due to their influence on nutrient dynamics. Stable isotope ratios of carbon, nitrogen, and sulphur were quantified to identify historical sources of organic matter. In addition, subfossil remains of diatoms were isolated from each section and taxonomically identified (minimum of 300 valves per sample). Preliminary results suggest that the diatom community in Lake Diefenbaker has typically consisted of planktonic species. Phosphorus loading and diatom community compositions have remained relatively constant in proximity to the Swift Current Creek outflow, suggesting a stable nutrient status over time. However, increased phosphorus loading and shifts in diatom community composition were observed in proximity to a large commercial fish farm, suggesting a trend towards increasing nutrient status. Future work with the three forms of sedimentary phosphorus, total sediment metals and stable isotope ratios will help to better understand nutrient dynamics and to trace organic matter sources within Lake Diefenbaker.

**WP148 Reproduction Toxicity to *Ceriodaphnia dubia*: “False Positives” Due to Epibionts** S. Clark, A. Briden, S. Ogle, Pacific EcoRisk; D. Schwartz, G. Cluster, M. Maidrand, A. Johnson, Sacramento Regional County Sanitation District. Numerous factors can affect a toxicity test, including the presence of non-target organisms (e.g., pathogens). In the mid-1990's, testing labs began reporting the presence of pathogen-related mortalities (PRM) in the chronic fathead minnow test, which resulted in the EPA's revision of the 2002 chronic testing manual to recognize and address PRM. However, potential pathogens are not limited to the fathead minnows. Recent microscopic examination of *Ceriodaphnia dubia* (exhibiting reduced reproduction) revealed the presence of epibionts (i.e., organisms

living on the surface of another organism), which were determined to be stalked ciliated protozoa. Food, detritus, and solids readily adhered to the epibionts' sticky stalks. The extremely rapid proliferation of the epibionts and the accumulation of particulates to the epibionts' sticky stalks resulted in the *Ceriodaphnia* becoming covered such that motility and molting appeared to be inhibited. Examination of the literature revealed that these epibionts compete with their hosts for food, and can cause reduced growth and reproduction for their host. Based on this information, the accumulation of these epibionts and the associated attached debris likely interfered with *Ceriodaphnia* feeding and ability to molt; the ability to molt is an essential precursor to the release of offspring. Without microscopic identification of the epibiont interference in the testing, routine analysis of the test data would have given a “false positive” for the reproduction endpoint. Multiple case studies will be presented in which the epibionts were observed on *Ceriodaphnia* exposed to samples collected from a WWTP with receiving water as the diluent, a tertiary treated effluent retained in ponds, an urban creek, and a creek influenced by agricultural runoff. The observation of these epibionts in multiple types of samples is consistent with the literature which indicates that they are known to be common in natural waters and also can occur in wastewater treatment plant (WWTP) biological treatment systems. Regulatory implications of the epibionts, and possible laboratory procedures/treatments to reduce epibionts will be discussed.

**WP149 Responses to mechanistically-related compounds in 2 pollution tolerant populations of *Fundulus heteroclitus*** J.T. Miller, A. Whitehead, University of California Davis; D. Nacci, U.S. EPA / ORD, NHEERL, Atlantic Ecology Division, U.S. EPA / NHEERL, Atlantic Ecology Division. Two mechanistically-related compounds were compared for their embryotoxic effects in populations of *Fundulus heteroclitus* (killifish) that have independently evolved resistance to dioxin like compounds (DLCs). While the exact mechanisms of tolerance are unknown and may vary for the pollution tolerant killifish populations that have been studied, DLC-tolerant killifish populations are all refractory to aryl hydrocarbon receptor (AHR) pathway activation. AHR-pathway suppression protects tolerant killifish from DLCs, which act solely through the AHR, but has mixed effects when tolerant killifish are exposed to compounds that act partly through the AHR, i.e., large mw PAHs. This study investigated the transcriptional responses to a prototypical DLC and large mw PAH of two killifish populations: one resident to a site highly contaminated with DLCs and the other to a site highly contaminated with PAHs. Embryonic response patterns will be used to infer mechanisms of tolerance and their implications for potential vulnerabilities and adaptive values associated with independently evolved-DLC tolerance in killifish.

**WP150 SETAC Advisory Group of International Experts on Water Quality Standards Development** U. Schneider, Patenta Inc / National Guidelines and Standards Office, Science and Risk Assessment Directorate; R. Wenning, ENVIRON. Water Quality Standards (WQS; also referred to as guidelines, criteria, benchmarks, thresholds, etc.) for the protection of aquatic life or aquatic ecosystems, an important tool in the management of environmental issues, are being developed by numerous jurisdictions around the world. Most of these jurisdictions use comparable approaches, but work independently of each other, resulting in duplication of work and effort. Even though some WQS developers know each other, and are in mostly informal contact, no official international network of WQS developers existed until recently. During the SETAC World conference in Berlin, Germany, in May 2012, about 20 international experts on the development of water quality standards created a SETAC Advisory Group on Water Quality Standards for the Protection of Aquatic Ecosystems. The experts came from government, academia, and industry from Europe, Asia, Australia, and North America. The primary goal of the Advisory Group is to be a formal network and resource center of WQS developers worldwide and to facilitate exchange of knowledge, experience, and expertise on WQS development. This poster describes the set-up, members, mandate, and goals of this advisory group.

**WP151 Standardizing and enhancing bioassessment protocols: Developing a science-based performance measure of stream condition** D.C. Lester, King County Department of Natural Resources / Water and Land Resources Division; J. Wilhelm, King County / Department of Natural Resources; L. Fore, Statistical Design; K. Adams, Washington Department of Ecology; G. Hayslip, EPA. Stream bioassessment protocols in the Puget

Sound region have been developed and applied by numerous local, tribal, and state jurisdictions since the early 1990s. Over 20 cities, counties, tribes and state agencies in the Puget Sound basin use a multi-metric benthic macroinvertebrate index (Benthic Index of Biotic Integrity – BIBI) to report stream health. However, a variety of factors made it difficult to compare and evaluate these data on a regional scale. Many entities use different sampling and analysis methods making data comparison challenging. The taxa attributes used to calculate the individual metrics that comprise the BIBI were inconsistent across the region and not empirically derived. The Puget Lowland BIBI was developed in the early 1990's based on a spatially limited data set causing concern about its regional applicability. The level of taxonomic resolution used by different entities was variable throughout the region. Lack of a regional data management system was also an issue, as well as the need for regional collaboration. The primary goal of this project was to evaluate differences in sampling methods, data analysis and data management protocols to develop standardized bioassessment methods to allow integration of monitoring and reporting across local and regional scales, in addition to enhancing regional collaboration between the numerous entities that assess streams using macroinvertebrate data. Data from more than 3,400 site visits at 950 sites were used to evaluate, recalibrate, and enhance the BIBI. Side by side samples were collected and analyzed to evaluate differences in sampling protocols. Sensitivity of taxa attributes was improved using empirical data and current science. The BIBI was rescored and recalibrated to reflect regional data and updated taxa attributes. The BIBI connects local jurisdictions to each other by providing a comparable measure of stream condition across watersheds and natural ecological gradients. The BIBI also connects regional and state programs, for example, the multimetric index is one of the 21 key ecosystem indicators selected by the Puget Sound Partnership for regional reporting. A standardized measure of stream condition allows regional prioritization for restoration and protection, provides a way to measure the effectiveness of management actions, and provides a science-based performance measure for streams. Methods and results from this effort will be presented.

**WP152 Statistical Analysis of Fish Early Lifestage Experiments** J.W. Green, DuPont / Applied Statistics Group. OECD TG 210 for fish early lifestage experiments is in the process of being updated. As part of that process, many GLP studies of freshwater and marine species have been re-analyzed under proposed revised guidelines and extensive computer modeling of both NOEC and ECx approaches have been done based on those studies. Responses to be analyzed under TG210 are size (length and weight), egg hatching success, larval mortality and abnormalities, first and last day of hatch, and first and last day of swim-up. It is the purpose of this presentation to present the results of these analyses and computer modeling studies, including specific hypothesis tests for NOEC determination and regression models for ECx estimation. It will be shown that regression provides good estimates of EC20 and EC30 in 75-90% of studies for size, hatch, and larval mortality, dependent on study conditions, but fails in 10-25% of studies for each of those responses. EC10 estimates are less often reliable, especially for hatching and mortality. NOECs are shown to be protective, even overly protective, in most studies for all responses except egg hatch and larval mortality, where the power to detect a 20% effect is less than 80% for the most variable species or data. Guidance will be given on statistical tests for each response and models for ECx estimation, as well as criteria by which to evaluate the quality of ECx estimates and NOEC determinations.

**WP153 The effects of methanol on echinoderm embryo-larval development, using the purple urchin *Strongylocentrotus purpuratus*** S. Douglass, H. Bailey, P. Arth, Nautilus Environmental; C. Stransky, A. Cibor, AMEC E&I; R. Kolb, City of San Diego. Testing the eluate of C18 solid-phase extraction columns is a commonly used treatment in toxicity identification evaluations (TIEs). This method involves passing a water sample through a C18 column in order to remove non-polar organic components, and then recovering this material through elution of the column with a solvent such as methanol. Toxicity recovered with this method provides a line of evidence that a non-polar organic compound is responsible for observed effects, and allows for magnification of the sample to double the original concentration or more. This method typically results in a solution with a methanol concentration of 0.8 percent. Testing of C18 column elutions has been required for multiple samples that have exhibited effects on the development of purple sea urchin (*Strongylocentrotus purpuratus*) embryos. A pilot study was therefore conducted to determine the sensitivity of

this species and endpoint to methanol in order to conduct the TIEs. Purple urchin embryo-larval development tests were conducted using methanol concentrations ranging from 0.031 to 2 percent. Embryos 12 and 24 hours post-fertilization were exposed to the same concentrations to evaluate the sensitivity of different early life stages to methanol. A threshold effect concentration of 1 percent methanol was determined for embryo-larval development. The development stage of the embryos exposed to methanol had little effect on sensitivity over the development period used. Results indicate that the purple urchin embryo-larval development test can be used successfully in TIEs that involve elution of C18 columns when methanol is used at typical concentrations.

**WP154 Using Fluorescent Nanoparticles within *Leptocheirus plumulosus* Tracking Microscopic Techniques to Assess Toxicity Mechanisms** G.L. McMennamy, M-I SWACO / Analytical; K. Nguyen, M-I SWACO / Quality Assurance; K.P. Hoelscher, M-I SWACO / Research. Understanding the factors that affect toxicity within benthic amphipods (*Leptocheirus plumulosus*) relevant to the oilfield is vital in order to maintain a safe environmental lifecycle surrounding the well site. Previous internal studies have introduced a technique to control variability observed in benthic sediment toxicity testing, by developing a health index to gauge the overall health of the organisms before test initiation to ensure standardization among test labs. Post test results showed some trending between the health index values and LC<sub>50</sub> results. To further understand why the amphipods died during a test, a forensic method was developed to detect aromatic and hydrocarbon based chemicals used in the test. Upon test termination, these chemicals were extracted from the animals system using a tissue extraction method. The extracted chemical quantification values correlated with the test toxicity results. During this research stage, the desire to track different molecules as these chemicals accumulate inside the organisms' bodies was noted. To approach this, an initial attempt must first be taken to understand where these chemicals may eventually end up inside the amphipods in order to understand the overall toxic effects. This study focuses on the ability to follow molecules within the organisms by exposing the amphipods to fluorescently tagged nanoparticles and tracking them with the aid of fluorescence microscopy. Images taken by the Zeiss Axio Imager Z2 Microscope illustrate evidence of fluorescence inside the test organisms, specifically within the digestive system and the body cavity. Some inherent fluorescence is apparent in control organisms when different regions of the body were examined under the microscope and noted. Various filters, magnification levels, and exposure time were also probed to maximize the optimal fluorescent difference between the groups. Uptake of fluorescently tagged silica nanoparticle by the organism did not show any toxicity effects on the organisms themselves. Fluorescent tagging of different chemistries may demonstrate the differences between the controls versus the test group on region-specific accumulation inside the organisms by qualitatively comparing images acquired by fluorescent microscopy.

**WP155 Use of PAM fluorometry in assessing field-derived periphyton community sensitivity to, and recovery from, herbicide exposure** R.S. Prosser, University of Guelph / School of Environmental Sciences; R. Brain, A. Hosmer, Syngenta Crop Protection; K. Solomon, University of Guelph / School of Environmental Sciences; M. Hanson, University of Manitoba / Department of Environment and Geography. Periphyton communities are an integral, yet under-appreciated, component of freshwater ecosystems, and in recognition of their foundational role, it is becoming more common to include periphyton in toxicity testing and risk assessment. The challenge is to develop robust methods to characterize effects on complex field-derived periphyton communities in the laboratory. This study investigated the feasibility of using field-derived periphyton to assess the toxicity of herbicides by measuring the quantum yield of photosystem II (PSII) with pulse amplitude modulated fluorometry. Periphyton samples were collected from select agricultural stream sites across the Midwestern United States in the summer of 2011. Periphyton was exposed to atrazine (10 to 320 µg/L) while PSII yield was measured at different time intervals (from 2 hr to up to 48 hr). Subsequently, the periphyton growth media was replenished (with control media) in order to assess recovery. Sensitivity to atrazine varied with site and date of sampling but EC10 and EC50 values for PSII quantum yield did not differ significantly with exposure interval. 75% of the periphyton samples exposed to 40 and 80 mg/L of atrazine had PSII yield values that were not significantly different from controls 2 to 6 hours after atrazine exposure. For the 25% of periphyton samples exposed to 40 and 80 mg/L of atrazine with

PSII yield values significantly different from the controls, the mean percent inhibition of PSII ranged from -42% (negative values indicates greater PSII yield than control) to 8%. Only the highest test concentration (320 µg/L) still demonstrated greater than approximately ~5% inhibition at 48hrs, however all other test concentrations exhibited recovery within ~5% of control levels. Rapid recovery of periphyton communities from atrazine exposure ( $\leq 24$  hr) was demonstrated with pulsed exposures. In 2012, sampling has been expanded to six sites and four time points.

**WP156 Using steady- and non-steady state assumptions to evaluate  $^{15}\text{N}$  and mercury bioaccumulation in lake trout** G. Paterson, R. Abma, K.G. Drouillard, D. Haffner, University of Windsor / Great Lakes Institute for Environmental Research. The stable isotope of nitrogen ( $\delta^{15}\text{N}$ ) has been proven to be a valuable predictor of anthropogenic pollutant bioaccumulation across multiple trophic levels of freshwater food webs. However, when evaluated at a single species levels,  $\delta^{15}\text{N}$  often becomes a less powerful predictor of pollutant bioaccumulation. Unlike persistent organic pollutants (POPs) such as polychlorinated biphenyls (PCBs) which bioaccumulate in the lipid phase of biota,  $^{15}\text{N}$  bioaccumulation is a function of animal protein content which also describes the primary tissue partitioning capacity for mercury (Hg) bioaccumulation. In this study, we used concentration metrics ( $\text{mg}^{15}\text{N}/\text{g}$  protein) and steady- and non-steady state assumptions to assess the bioaccumulation of  $^{15}\text{N}$  and Hg in multiple ages (1-13 yrs) of lake trout collected from Lake Huron in 2011. Specifically,  $^{15}\text{N}$  concentrations exhibited no age related increases when normalized to animal protein content estimated as lean dry weight. In contrast, Hg concentrations demonstrated a significant age related increase in this protein fraction of lake trout. Under the steady state assumption, these observations suggest that  $^{15}\text{N}$  elimination kinetics are substantially different from those of Hg in fish tissues and may help to explain the general lack of correlation between  $\delta^{15}\text{N}$  and anthropogenic pollutants at the single species scale. Further, we propose that variability in  $\delta^{15}\text{N}$  trophic enrichment factors ( $\Delta\delta^{15}\text{N}$ ) is associated with differences in protein content between predator and prey. In this manner,  $\Delta\delta^{15}\text{N}$  values may reflect protein content normalized  $^{15}\text{N}$  bioaccumulation factors (BAFs) that are similar to the lipid normalized BAFs used to assess POP bioaccumulation in freshwater food webs.

**WP157 Assessment of polybrominated diphenyl ethers and 2,2',4,4',5,5'-hexabromobiphenyl in bottom sediment of some effluent-receiving Rivers in Cape Town** O.S. Fatoki, Cape Peninsula University of Technology / Department of Chemistry; J.P. Odendaal, Cape Peninsula University of Technology / Department of Environmental and Occupational Studies; A.P. Daso, Cape Peninsula University of Technology / Department of Food and Agricultural Sciences. Until recently, studies reporting the environmental levels of polybrominated diphenyl ethers and polybrominated biphenyls in Africa are rarely found in the literature. Consequently, this study was aimed to assess the concentrations of commonly investigated PBDE congeners (BDE 28, 47, 100, 99, 154, 153, 183 and 209) as well as BB 153 in bottom sediment of two rivers receiving effluent discharges within the City of Cape Town. In this study, both GC- $\mu$ ECD and GC-TOF-MS techniques were employed for the routine analysis and the structural elucidation of the target compounds, respectively. The overall mean concentrations of the total PBDE ranged from 0.06 – 2.47, 0.07 – 0.58 and 0.10 – 0.78 ng/g for the upstream, point of discharge and downstream sampling points, respectively. These values were relatively higher in the Kuils River samples where the overall mean concentrations were found to be 0.22 – 1.52, 2.36 – 9.95 and 0.31 – 5.38 ng/g for the upstream, point of discharge and downstream sampling points, respectively. The average levels of BB 153, which were generally low, ranged from ND – 0.33, ND – 0.22 and 0.04 – 0.39 ng/g for the upstream, point of discharge and downstream sampling points, respectively. Similarly, the average levels of BB 153 in the Kuils River were in the range of ND – 0.18, ND – 1.01 and ND – 1.21 ng/g for the upstream, point of discharge and downstream sampling points, respectively. The statistical analyses performed on these data showed that majority of the PBDE congeners, except BDE 209 had strong positive correlation with the total organic carbon (TOC). Furthermore, the Pearson's correlation analyses conducted amongst the congeners showed that majority of the PBDE congeners as well as BB 153 had strong positive relationship with one another, which were statistically significant at both 0.01 and 0.05 (p-values), thus suggesting the possible similarity in the sources of these pollutants into the investigated rivers. This study further confirms the potential of wastewater

treatment plants' discharges as important source of PBDEs in the receiving environment.

**WP158 A Study of the In Vitro Bioaccessibility of Nickel Compounds in Particulate Form as Affected by Variation in Assay Conditions** W. Lau, B. Hale, University of Guelph / School of Environmental Sciences; M. Dutton, Vale Ltd.. Abiotic tools for evaluating oral bioavailability of Ni from metal-contaminated soils rely on chemical extractions to determine the fraction of Ni that would be liberated in biological fluids. Bioaccessibility is an artificial concept that considers only the dissolution phase of the digestion process, and is determined using *in vitro* assays. Oral bioaccessibility of metals in soils has been assessed by a variety of *in vitro* methods. Bioaccessibility assay conditions have been demonstrated to influence the percent of total metal leached from soil. The use of bioaccessibility data in a site-specific risk assessment requires regulatory acceptance of the data which is usually dependant on scientific validation of the test method. In this respect, it is fundamentally important to consider the mineralogical controls of the *in vitro* dissolution of the various forms of Ni emitted from industrial processes. A series of experiments were conducted which compared *in vitro* bioaccessibility for a number of pure Ni compounds ( $\text{NiO}$ ,  $\text{NiSO}_4$ ,  $\text{Ni}_3\text{S}_2$ ,  $\text{NiS}$ , Ni metal) in particulate form, as affected by variations in two method parameters: extraction ratio (volume of extractant to mass of nickel), and digestion time. In general, the extraction of Ni was found to be different among nickel compounds. Bioaccessible Ni increased with extraction ratio and digestion time for most compounds. However, *in vitro* extractions for  $\text{NiO}$  returned relatively lower values that were negatively correlated with increased extraction ratio. Normalizing bioaccessibility estimates for particle shape and specific surface area, did not explain the observed differences. Rather, the anomalous behaviour of  $\text{NiO}$  is likely a surface-access solubility limitation which is causing the reduced bioaccessibility of  $\text{NiO}$  at lower extraction ratios. For *in vitro* data to have greater practical uses in risk assessment models, there is a need for further optimization of method parameters. Pure compounds provide a base scenario for comparison with Ni species that are present in combination with soil. Investigations of additional Ni species in particulate form can provide the data required to validate particular *in vitro* methods and approaches, with respect to result consistency vs. method dependency in observations.

**WP159 Accumulation of 2,4,6-trinitrotoluene adducts in the freshwater snail, *Planorbella trivolvis*, and the potential for use as a biomarker of exposure** K. Sieve, J. Belden, Oklahoma State University / Department of Zoology. Explosive compounds have been extensively released into the environment for decades through manufacture and during disposal at military sites, which has lead to contamination of soils, ground waters, and surface waters. This contamination is of environmental concern, especially in situations of continuous release from unexploded ordnance. Although the nitroaromatic compound 2,4,6-trinitrotoluene (TNT) is predicted to have a low bioaccumulation potential ( $\log K_{ow} = 1.6$ ), TNT is reactive in live animal tissues and readily binds to macromolecules, forming residues that are persistent and difficult to extract with standard solvents (nonextractable residues). These nonextractable residues may serve as biomarker for TNT exposure if analytical procedures can be developed. Aquatic snails are a potential species to use as a biomonitoring organism since many snails tend to spend their entire lives within a few square meters and tend to accumulate contaminants. To determine if TNT forms nonextractable residues in aquatic snails and to determine the degree of accumulation, *Planorbella trivolvis* were exposed to radiolabelled ( $^{14}\text{C}$ ) TNT-spiked water for 96 hours. Nonextractable residues accounted for  $37 \pm 4\%$  of the total recovered radioactivity. The biological half-lives of the fractions were very similar. Studies are currently being conducted to determine if a GC/MS method is able to identify compounds without a radiotracer. Assessing environmental exposure to TNT can be challenging due to its transient nature; however, the identification of nonextractable residues may point to a more specific biomarker that can be used to assess TNT contamination.

**WP160 Application of a Linked QWASI+AQUAWEB model for Estimating and Evaluating Bioaccumulation of Hydrophobic Compounds in Aquatic Food Webs** J. Kim, Dow Corning Corporation / Health and Environmental Sciences, Dow Corning Corporation / Dow Corning Corporation; D.E. Powell, Dow Corning Corporation / Health & Environmental Sciences, Dow Corning Corporation / Health & Environmental Sciences (C03101); K.B. Woodburn, Dow Corning Corporation / Health



& Environmental Sciences; R.M. Seston, Dow Corning Corporation / Health & Environmental Sciences, Dow Corning Corporation / Health and Environmental Sciences; F.A. Gobas, Simon Fraser University / School of Resource and Environmental Management (Faculty of Environment), Simon Fraser University / School of Resource & Environmental Management, Faculty of Environment, Simon Fraser University. Assessment of the potential for chemicals to accumulate in living organisms (bioaccumulation) and to increase in concentration with increasing trophic level within aquatic food webs (biomagnification) are integral aspects of the risk assessment process. Bioaccumulative substances are defined as those substances that bioaccumulate in organisms and biomagnify in food webs. The trophic magnification factor (TMF) describes the accumulation and increase in concentration of a chemical that occurs in organisms that occupy successively higher trophic levels within a food web. It has been proposed that  $TMF > 1$  may be the best indicator of a bioaccumulative substance. Models developed to evaluate bioaccumulation and biomagnification of chemicals in defined aquatic food chains are used in the United States and Canada for risk assessment and to identify potential bioaccumulative substances. However, these models rely on static concentrations to estimate chemical exposure in water and sediment, which does not take into account dynamic and variable conditions of exposure that may occur in aquatic systems. TMF values may be skewed by variable inputs of a chemical into a food web, concentration gradients, localized hot spots of chemical exposure, or by migrating species that are exposed to different conditions than organisms in the local food web. These are especially relevant considerations when evaluating the TMF of chemicals having point-source emissions. To demonstrate and correct for these biases, the multimedia fugacity model QWASI (Quantitative Water Air Sediment Interaction) and the bioaccumulation model for aquatic food webs AQUAWEB were coupled together. This was possible by employing a newly developed QWASI-Excel model that functions on the same spreadsheet platform as the AQUAWEB model. The linked models allow a chemical to be released into a defined aquatic system within QWASI, which calculates the distribution and concentrations of the chemical in sediment and water for each compartment or segment of the defined system. The output from QWASI is then used by AQUAWEB to calculate concentrations in aquatic organisms of the defined food web for each compartment. The linked QWASI+AQUAWEB models are especially useful for evaluating bioaccumulation in food webs of heterogeneous systems where migration or variable exposures may occur or that are confounded by concentration gradients—e.g., concentration gradients across the sediment/water interface.

**WP161 Arsenic speciation of various aquatic organisms collected from Pohang and Ulsan industrialized areas of Korea** S. Hong, Korea University / Division of Environmental Science and Ecological Engineering; J. Khim, Korea University / Division of Environmental Science and Ecological Engineering, Korea University / Environmental Science and Ecological Engineering; J. Park, Korea University / Environmental Science and Ecological Engineering; H. Son, S. Choi, Ulsan National Institute of Science and Technology (UNIST) / Environmental Analysis Center. We determined the bioconcentration and species-specific distribution of Arsenic (As) compounds in various aquatic organisms collected from the industrialized areas of Pohang and Ulsan, South Korea. Total of 58 biota samples including fish (n=24), bivalve (n=13), crab (n=5), shrimp (n=7), gastropod (n=7), and algae (n=2) were collected from Pohang and Ulsan in 2010. Six As species including arsenobetaine (AB), arsenochlorine (AC), arsenite ( $As^{III}$ ), arsenate ( $As^V$ ), monomethylarsinic acid (MMA), and dimethylarsinic acid (DMA) were separated and quantified using HPLC-ICP/MS. All biological samples contained As compounds, and the measured concentrations in biota generally reflected the degree of contamination for waterborne and sedimentary As. The greatest concentration (42  $\mu\text{g/g dw}$ ) of total As was found in marine algae (*Enteromorpha*), followed by crab, bivalve, and shrimp, on average. Concentrations of As in fish samples (0.59 – 11  $\mu\text{g/g dw}$ ) were generally less than those in other biota samples. Composition of As species in biological samples greatly varied both within and among the group of species. AB was found to be the most dominant species (viz. compound) among 6 As species in fish, while not only AB but also  $As^{III}$  were abundant in bivalve, crab, and shrimp. In gastropod, most toxic arsenic,  $As^{III}$ , was shown to be the predominant species. It is suggested that composition of As species in various aquatic organisms would be associated with their metabolism of As. Correlation analysis indicated that significant relationship between As concentrations of water and biota for fish, bivalve, and shrimp species. However, there was no correlation found in case of crab and gastropod,

probably due to the differences in feeding behaviors among the species. Further, organ-specific analysis enabled us to find the biotransformation of As in fish (gill, intestine, and fillet) and bivalve (siphon, intestine, mantle, and foot). The result indicated the detoxification pathways from inorganic to organic As. Overall, the concentration of toxic As (viz. inorganic As) found in biota samples in the present study was generally smaller than that of non-toxic As. However, it should be noted that toxic As species contributed about 43% of total As concentrations in gastropod, which should be further determined in terms of site-specific and/or species-specific bioaccumulation.

**WP162 Bioaccumulation of trace metals in the blue crab *Callinectes danae* from the Santos Estuarine System, Brazil** L.C. Bordon, J.E. Sarkis, M. Lima, M.A. Hortellani, N.P. Andrade, IPEN – Nuclear and Energy Research Institute / Center for Chemical and Environmental Technology; A.R. Tomas, A. Scalco, Fisheries Institute – Sao Paulo State. This study is an assessment of trace metals in tissues of the blue crab *Callinectes danae*, aiming to confirm the metal bioaccumulation process in this species. In August/2011, 82 blue crabs were collected along the Santos Estuarine System, in Sao Paulo State, Brazil. Muscles, hepatopancreas and gills were removed by dissection and trace metals were measured in these three types of tissue. Al, Cd, Cr, Co, Mn, Ni and Pb concentrations were determined by HR-ICP-MS whereas Cu, Fe and Zn concentrations were measured using flame mode of a Fast- Sequential Atomic Absorption Spectroscopy. Particularly, Hg concentration was measured by cold vapor generation. The concentration of Hg was higher in muscles whereas hepatopancreas presented higher concentrations of Cd, Co, Cr, Ni and Zn. The concentrations of the other metals were higher in gills. In the Discriminant Analysis, Cu and Zn presented the highest coefficients and contributed most to the discriminatory power of function I, distinguishing muscles and gills samples. The coefficient of Ni contributed to the power of function II, distinguishing muscles and hepatopancreas samples. The results are in agreement with previous studies, confirming the bioaccumulation process in the blue crab *Callinectes danae*.

**WP163 Bioaccumulation, Tissue distribution and Maternal Transfer of Pharmaceuticals and Personal Care Products in Zebrafish (*Danio rerio*)** E. Chen, National University of Singapore / Department of Civil and Environmental Engineering; Z. Gong, National University of Singapore; B.C. Kelly, National University of Singapore / Department of Civil and Environmental Engineering. The occurrence of pharmaceuticals and personal care products (PPCPs) in the environment has received increasing interest in recent years. The bioaccumulation behavior of PPCPs in aquatic organisms is not well documented. This study involves laboratory investigations to assess the bioaccumulation behavior of PPCPs in zebrafish (*Danio rerio*). We conducted a continuous flow-through exposure experiment to assess the bioaccumulation kinetics, tissue distribution and maternal transfer of several PPCPs in adult female zebrafish. Bioconcentration experiments involved 28 days of aqueous exposure, followed by a 14-day depuration phase, at high and low exposure concentrations. For exposure experiment, fish were collected at six time-points during uptake phase and five time-points during the depuration phase. Gills, liver, muscle, blood and eggs were collected from individual fish and pooled into composite samples (five fish per composite). The tissue/biofluid samples were extracted and cleaned up by sonication and solid phase extraction (SPE). Determination of test compound concentrations was conducted by analysis using liquid chromatography tandem mass spectrometry (LC-MS/MS). In addition, *in vitro* hepatic microsomal assays utilizing microsomes isolated from zebrafish liver tissue were conducted to assess metabolic transformation of PPCPs in zebrafish. Observed bioconcentration factors (BCFs) varied among test compounds and ranged from approximately 15 to 500 for the various PPCPs investigated. *In vitro* hepatic microsomal assays demonstrated moderate to high biotransformation potential for several PPCPs in zebrafish liver microsomes, which likely caused some of the variability in observed BCFs. The results are further evaluated to assess the role of key biological constituents (proteins, phospholipids) and influence of octanol-water and protein-water distribution coefficients (Dow, Dpw) on bioaccumulation potential of PPCPs in aquatic organisms.

**WP164 Butyltin compounds in estuarine catfishes (*Siluriformes*, *Ariidae*) after TBT banning** D.M. Santos, Sao Paulo University, Sao Paulo University / Chemical Oceanography; M. Rodrigues de Marchi, São Paulo State University; A. Turra, University of São Paulo; R. Carmela Montone, São Paulo State University. Estuarine environment and the fishes associated are potentially affected by human actions getting in risk food resources,

distribution, diversity, reproduction, growing and behavior of residents and migratory fish species. Due their wide application in several human activities, butyltin compounds (BTs) were considered as the most deliberately introduced contaminants into the environment and, despite the antifouling TBT ban in 2008, they can be found in all aquatic compartments. In Brazil, since 2001, many studies have been developed focusing abiotic and biotic matrices and the results still worrisome. Studies involving organisms demonstrate a bioaccumulation through sediment/water uptake or even by thropic chain bring a serious ecological risk for these communities. Butyltin impact was investigated in 8 estuaries at Brazilian coast, including harbor and preserved areas to access the impact of this compounds in three catfish species: *C. spixii*, *A. luniscutis* and *G. genidens*. The accumulation of BTs in liver and gills ranged from n.d. to 1426 ng g<sup>-1</sup> and n.d. to 218 ng g<sup>-1</sup> respectively, among estuaries and species. *G. genidens* has shown higher uptake of these compounds related with their dislocation through estuary for feeding and reproduction, keeping in contact with the main TBT punctual sources. Among the investigated estuaries, Itajaí-Açu Estuary located at Brazilian south coast presented the highest BTs levels for all compounds, demonstrating a recent input even after TBT banning. The results have shown that besides abiotic matrices these compounds can be detected in estuarine fishes, showing the bioavailability of these pollutants and bring a real impact for local biota and ecosystem beyond human riverine communities that use these species as protein source.

#### WP165 Comparing Estimated and Empirical Fish Bioconcentration

**Values** A. Samel, DuPont Crop Protection / Ecotoxicology; S. Loutseti, DuPont Crop Protection; D. Nabb, R. Mingoia, DuPont Haskell Global Centers for Health and Environmental Sciences. The purpose of the fish bioconcentration test (OPPTS 850-1730., OECD 305) is to determine if a chemical will accumulate in the edible and non-edible fractions of fish. The test is labor intensive, very expensive, and may require large numbers of fish for the pilot and definitive studies. *In silico*, *in vitro*, and reduced fish bioconcentration test methodologies have been developed as potential tiered alternatives to the guideline fish bioconcentration test. However, regulatory acceptance of these alternative methodologies has lagged because of a lack of data to correlate the endpoints from the alternative tests to the bioconcentration value determined from the guideline test. We will present data from the alternative methods and the guideline study for a new chemical, including a profile of the metabolites identified in the guideline test. The results indicate that for well characterized chemical compounds/classes it is possible to calculate a reasonable bioconcentration factor for regulatory purposes using alternative methodologies and thereby preclude the need for a full OECD/OPPTS fish bioconcentration test and its associated use of resources, including test organisms.

#### WP166 Comparison of in vitro estimates of bioaccessible Ni in field-contaminated soils and identification of mineralogy

L. Vasiluk, University of Guelph / Department of Land Resource Science, University of Guelph / School of Environmental Science; M. Dutton, Vale Canada Limited; B. Hale, University of Guelph / School of Environmental Sciences. The bioavailability of a contaminant mixed with the soil matrix depends upon the soil properties, the contaminant and the manner by which the contaminant entered the soil. This study considers soil Ni contamination around smelting and refinery sites, such as the Vale complex in Sudbury (Sud) and Port Colborne (PC), ON, Canada. The objective was to determine if there is a relationship among particle size, trace element bioaccessibility and mineralogy, as the mineral form of the metal is a critical determinant of bioaccessibility/bioavailability. At each location, two naturally weathered soils were collected and separated into two fractions (150-250µm and < 70µm) with elevated Ni concentrations from historic Ni emissions. Identifying the mineralogy was achieved with a non-specific sequential extraction using increasing strengths of nitric acid followed by chemometric data processing (CISED), SEM-EDS and XANES Spectroscopy. Once the predominant minerals in the soil associated with Ni were identified, its relative chemical "solubility" was compared to the *in vitro* assay (PBET). In the Sud soils, the smaller particle fractions, compared to the larger, had the greatest Ni concentration; in one soil the effect was 8 fold while the other soil was a 3 fold difference. In the PC soils, total Ni concentrations were not different between the fractions but compared to the Sud soils, had higher soil Ni. Total Ni concentrations in PC soils were not different between the fractions, but much higher than in Sud soils. The comparison of bioaccessible Ni (PBET) results and cumulative Ni from CISED, showed that PC soils reached the

PBET estimates for easily extractable Ni at a lower nitric acid concentration (up to 0.01M), while Ni from Sud soils required higher strength nitric acid. Chemometric analysis identified a different number of physico-chemical soil components for each soil or its fractions. The majority of Ni was co-eluted with the "carbonate" component, extracted mainly in 0.01-0.05 M HNO<sub>3</sub>, and was made up principally of Ca, Mg and Al in addition of Na, K and Cu. The high proportion of Ca suggests that these components could be carbonate material in a humic acid exchangeable fraction or clay-like matrix if Si is present. The mineralogy of the soil fractions from the same site was similar, but different between the two sites: PC soils were dominated by NiO whereas Ni-bearing particles in the Sud soils were FeNiO. Neither of these mineral forms of Ni is very soluble.

#### WP167 Coupling Transcellular Permeability and Hepatic Microsomal Biotransformation Assays for Assessing Bioaccumulation Potential of Commercial Chemicals

Y. Wang, J. Koh Cai Fen, B.C. Kelly, National University of Singapore / Department of Civil and Environmental Engineering. The Stockholm Convention on Persistent Organic Pollutants (POPs) aims to reduce or eliminate the production of persistent, bioaccumulative and toxic (PBT) chemicals. There is a current need to develop effective *in vitro* assays for screening existing and newly manufactured commercial chemicals in order to better identify those substances that can persist in the environment, bioaccumulate in food chains and cause adverse effects in wildlife and humans. This study presents results of two *in vitro* assays for assessing the toxicokinetics and bioaccumulation potential of commercial chemicals. In particular, we conducted a parallel artificial membrane permeability assays (PAMPA) to assess passive transcellular permeability and hepatic microsomal assays utilizing microsomes isolated from fish liver tissue to assess metabolic transformation of emerging contaminants. Test chemicals included legacy POPs (polychlorinated biphenyls, organochlorine pesticides), as well as several emerging contaminants of concern, including current use pesticides (CUPs) and pharmaceuticals and personal care products (PPCPs) and brominated flame retardants (BFRs). The developed PAMPA assays, which involved 96 hour controlled chemical transport between stationary donor and acceptor PDMS disks in solution, were successfully used to quantify apparent permeability (LogP<sub>app</sub>) of test compounds. Observed LogP<sub>app</sub> values (range: 4.49E-07 to 2.8 E-09 cm/s) were negatively correlated with chemical's octanol-water partition coefficient (Kow), over a log Kow range of 3 to 10. Hepatic microsomal assays were used to quantify biotransformation of legacy POPs and emerging contaminants of concern. The extent of chemical biotransformation was variable among compounds and between fish species. Legacy POPs exhibited negligible biotransformation rates in fish liver microsomes. Musk Xylene, a nitro musk fragrance compound, exhibited the least amount of metabolic transformation, with only 1-10% of the compound being degraded in these fish liver microsomes. Other emerging contaminants exhibited metabolism to varying degrees in the hepatic microsomal biotransformation assays. The developed PAMPA and hepatic microsomal biotransformation assays provide a simple and cost-effective approach for eliciting key toxicokinetic data.

#### WP168 Development of Membrane Equipped Isolation Chambers for Assessment of Infaunal Invertebrate Exposure to PCBs via Sediment Porewater

J.G. Coleman, US Army Engineer Research and Development Center, US Research and Development Center / Environmental Laboratory, US Army Engineer Research and Development Center / Research Biologist, US Research and Development Center / Research Biologist; A.J. Kennedy, US Army Engineer Research and Development Center, CEERD-EPR; G. Lotufo, US Army Engineer Research and Development Center / Environmental Laboratory; C. Ruiz, USACE – ERDC; T.S. Bridges, US Army Engineer Research and Development Center, U.S. Army Corps of Engineers / USERDC-WES-EM-D. Remediation of contaminated sites based on bioaccumulation assessments of benthos is often overly conservative due to generalizations that arbitrarily group organisms into a single category. This practice fails to consider exposure relevance or functional ecology. The present study quantifies realistic bioavailability assessments to infaunal invertebrates through development of pathway isolation chambers for assessing chlorinated hydrocarbons exposure in sediment porewater. We tested four membranes at large and small pore sizes (0.1 – 1.2 µm nylon, polycarbonate, polyethylsulfone, and polytetrafluoroethylene with a hydrophilic coating) to determine their potential to allow chlorinated hydrocarbons contamination into a pathway isolation exposure chamber while excluding dissolved organic carbon (DOC). Initially, we conducted a water-only 60-hour passive



diffusion study to assess potential for  $^{14}\text{C}$  dichlorodiphenyltrichloroethane (DDT) to diffuse across each membrane fitted to a glass vial, followed by a similar set-up with  $^{14}\text{C}$ -DDT and  $^3\text{H}$ -fluoranthene spiked into a sediment slurry with continuous suspension. Diffusion was measured with a liquid scintillation counter (LSC). Based upon diffusion of chlorinated hydrocarbons within each vial, the Teflon membrane performed best overall for maximum diffusion with minimal sorption. Studies with 50 mg/L of humic acid spiked into 30 ppt seawater were conducted to determine diffusion potential across 0.1 and 1.0  $\mu\text{m}$  Teflon membranes, although, neither membrane effectively excluded the dissolved humic matter. An additional exposure was conducted utilizing all glass chambers covered with the 1.0  $\mu\text{m}$  Teflon membrane containing solid-phase microextraction fibers (SPMEs) deployed in the sediment and within the chamber after 7, 21, 28, 56-days. Results indicated an upward trend for PCB levels relative to time and confirmed success of the Teflon membrane for a whole sediment system. Ultimately, the Teflon membrane was selected for use in a larger exposure system to construct pathway isolation chambers exposing *Mercenaria mercenaria*, *Leptocheirus plumulosus*, *Neanthes arenaceodentata*, and *Eohaustorius estuaries* to field collected contaminated sediments to gain a better understanding of how functional feeding behavior strategies can factor into exposure. This research is expected to reduce uncertainty associated with current exposure assessments and predictions used in remediation selection and design.

**WP169 Dietary exposure bioaccumulation fish test using two fish species (common carp and rainbow trout)** T. Sawada, N. Hashizume, Y. Inoue, T. Yoshida, H. Murakami, Y. Suzuki, Y. Koga, R. Takeshige, N. Hario, A. Tanabe, Y. Kawashima, T. Ishibashi, E. Kikushima, Chemicals Evaluation and Research Institute, Japan. The potential for chemicals to bioaccumulate in organisms is a key element in chemical environmental risk assessment. Existing regulatory criteria for bioaccumulation assessment of chemicals are mainly based on a bioconcentration factor (BCF) not a biomagnification factor (BMF). The Organization for Economic Cooperation and Development (OECD) is currently considering the adoption of a testing method for poorly water-soluble chemicals using a novel dietary exposure technique to estimate BMF according to the OECD test guideline (TG) 305. In TG 305, several fish species are recommended to estimate bioaccumulation potential of chemicals; however, one possible source of the observed variability in bioaccumulation potential between fish species is the metabolism and biotransformation of chemicals in the fish. We selected five aromatic compounds with an array of presumed bioaccumulation potential (hexachlorobenzene [HCB], musk xylene, *ortho*-terphenyl, methoxychlor [MXC], benzo[a]pyrene [BAP]) and compared the BMF parameters of two fish species—common carp (*Cyprinus carpio*) and rainbow trout (*Oncorhynchus mykiss*). The lipid-corrected BMFs of HCB between the two fish species were almost the same values (common carp, 1.7; rainbow trout, 1.8); however, those of MXC were quite different (common carp, 0.012; rainbow trout, 0.36) and BAP was not detectable in fish tissues at the early depuration phase (common carp, < 0.05  $\mu\text{g/g}$  on day one of the depuration phase; rainbow trout, < 0.004  $\mu\text{g/g}$  on day eight of the depuration phase), indicating that common carp may have a greater capacity to biotransform certain organic chemicals than rainbow trout. We conclude that HCB might generally not be metabolized by fish and not affect the metabolic capacity of the fish. Care should be taken to check the fish species as test organisms when estimating the bioaccumulation potential of highly metabolizable substance like MXC and BAP. For these chemicals, it may also be advantageous consider setting a longer uptake duration or a higher dietary concentration.

**WP170 Exploring A New Screening Technique For Assessing the Bioconcentration Potential of Pharmaceuticals in Fish** L.A. Constantine, Pfizer Global Research and Development / Pharmacokinetics, Dynamics & Metabolism, Pfizer, Inc. / Pharmacokinetics, Dynamics and Metabolism, Pfizer, Inc. / Chemical Research & Development; D.B. Huggett, University of North Texas / Department of Biological Sciences, University of North Texas / Institute of Applied Sciences. Understanding whether an active pharmaceutical ingredient is likely to be taken up from the aquatic environment by fish and bioaccumulate has triggered the need to conduct bioconcentration studies in fish. As per EMEA Guidelines, a bioconcentration study is required in the PBT assessment for pharmaceuticals having a  $\text{Log } K_{ow} > 4.5$  and in the Phase II, Tier A assessment for pharmaceuticals having  $\text{log } K_{ow}$  values > 3. The standard OECD Guideline 305 includes an exposure (uptake) phase followed by a post-exposure (depuration) phase typically equal to 1/2 the duration of the uptake phase. Based on the sampling

schedule outlined in the guideline, >100 fish per study may be required to determine a kinetic BCF value. Based on the resources required to conduct the full bioconcentration study and the potential for fish to metabolize xenobiotics, alternatives to the current bioconcentration tests as a first tier have been explored and employed for the last several years. An abbreviated protocol including up to 7 days of uptake and 7 days of depuration has been conducted in the zebrafish with several pharmaceuticals. The BCF values obtained from these studies will be compared to the full OECD 305 BCF values to support use of the 14 day study as a screening tool to assess the bioconcentration potential in fish.

**WP171 Fugacity: The Rosetta Stone for Interpreting Bioaccumulation and Biomagnification in Aquatic Environments** D.E. Powell, Dow Corning Corporation / Health & Environmental Sciences, Dow Corning Corporation / Health & Environmental Sciences (C03101); K. Kidd, Canadian Rivers Institute, University of New Brunswick / Biology, University of New Brunswick / Biology department; D. Mackay, Trent University, Trent University / Environmental Studies; R.M. Seston, Dow Corning Corporation / Health & Environmental Sciences, Dow Corning Corporation / Health and Environmental Sciences; K.B. Woodburn, Dow Corning Corporation / Health & Environmental Sciences. The potential of a chemical to accumulate in living organisms (bioaccumulation) and increase in concentration with increasing trophic level within a food web (biomagnification) are important considerations for assessing ecological risk. Measures used to assess bioaccumulation and biomagnification take into consideration that pathways of exposure may occur through various sources (i.e., water, sediment, diet, and air). For aquatic organisms, bioconcentration factors (BCF; units of L/kg lipid) describe the uptake and accumulation of chemicals from water only. Bioaccumulation factors (BAF; units of L/kg lipid) describe uptake and accumulation from all sources (e.g. water, sediment, diet, etc.) relative to the amount of chemical stored in the water compartment. Similarly, biota-sediment accumulation factors (BSAF; units of kg OC/kg lipid) describe uptake and accumulation from all sources relative to the amount of chemical stored in the sediment compartment. Biomagnification factors (BMF; non-dimensional units of  $C_{\text{predator}}/C_{\text{prey}}$ ) describe the increase in concentration of chemicals in organisms that are separated by a single trophic level step on a food chain. Similarly, trophic magnification factors (TMF; units of  $C_{\text{organism}}/\text{trophic level step}$ ) describe the increase in concentration of chemicals in organisms that occupy successively higher trophic levels within a food web. Regulatory screening and assessment criteria used to identify potential bioaccumulative and biomagnifying substances are typically based on laboratory measurements of BCF or field measurements of BAF and BSAF. However, the field-based TMF has been proposed as a preferable metric and is increasingly being used by researchers to assess bioaccumulation and biomagnification of chemicals in the environment. This has resulted in spirited debate on the utility and comparability of the various metrics for bioaccumulation (BCF, BAF, BSAF) and biomagnification (BMF, TMF). This presentation will show how these metrics are interrelated when expressed in equivalent terms of fugacity (units of Pa) and fugacity ratios (non-dimensional units of Pa/Pa). These interrelationships will be applied to field data for select contaminants and used to demonstrate how to minimize or eliminate bias and uncertainty associated with nitrogen-15 enrichment factors (used to estimate relative trophic level position), omnivorous feeding, migration, and exposure to concentration gradients.

**WP172 Impact of Kingston fly ash on freshwater mussels: a combination field and laboratory approach** R.R. Otter, Middle Tennessee State University / Biology, Middle Tennessee State University / Department of Biology, Middle Tennessee State University / Assistant Professor; W. Monroe, Middle Tennessee State University; D. McKinney, B. Brown, S. Lainer, Tennessee Wildlife Resources Agency; B. Read, Tennessee Department of Health. On December 22, 2008 the largest release of fly ash in United States history occurred at the TVA Kingston Fossil Plant in Roane County, Tennessee. The result was contamination of the Emory River and surrounding area. Efforts to understand the toxicological implications of this spill are currently underway with investigations into the impact of fly ash on a wide range of biota. In this study we investigated the impact of Kingston fly ash on freshwater mussels, in both a field and laboratory setting. Our field study consisted of two consecutive years of in situ exposure of five different native freshwater mussel species at both upstream and downstream sampling sites, compared to the location of the spill. Endpoints used to determine impact included the bioaccumulation of 23 metals, (including mercury,



arsenic, and selenium), a mussel health index, and glycogen content in multiple tissues. Results were species dependent, but overall, all species showed a high survivability throughout the entire exposure period and very low bioaccumulation of metals compared to reference mussels. A laboratory exposure using *Utterbackia imbecillis* was also performed using a custom static recirculating tank design to expose freshwater mussels to varying concentrations of Kingston fly ash over a six month duration. Bioaccumulation of metals and glycogen concentrations were measured and compared directly to the treatment concentration of fly ash. Results varied for each metal based on exposure time and ash concentration. All mussels (across all time points and fly ash exposures) concentrations of lead and selenium were found to be below detection limit, while arsenic was found to be influenced by both time and fly ash exposure with a maximum body burden of 18.75 mg/kg dw (6 month exposure to 100% fly ash) compared to reference organisms at the same time point which had an average concentration of 6.5 mg/kg dw.

**WP173 Importance of lipid analysis and its implications for metrics of bioaccumulation** R.M. Seston, Dow Corning Corporation / Health & Environmental Sciences, Dow Corning Corporation / Health and Environmental Sciences; D.E. Powell, Dow Corning Corporation / Health & Environmental Sciences, Dow Corning Corporation / Health & Environmental Sciences (C03101); K.B. Woodburn, Dow Corning Corporation / Health & Environmental Sciences; H. Gandhi, Michigan State University / Department of Zoology; P.W. Bradley, Michigan State University / Department of Animal Science; M.J. Zwiernik, Michigan State University / Department of Animal Science, Michigan State University, Wildlife Toxicology Laboratory / Department of Animal Science, College of Veterinary Medicine. For the quantification of chemical residues in environmental matrices, many standardized methods have been developed. These methods commonly include rigorous quality control programs to ensure the accuracy and precision of the results. However, the accuracy and precision with which lipid determinations are performed are not held to the same level of rigor as that associated with analysis of chemical residues. Accurate measures of lipid content are of great importance as chemical residues are commonly normalized to lipid content so that results are more comparable between different tissues and different organisms. Additionally, lipid-normalized concentrations of chemical residues are required to calculate fugacity and may be used to calculate measures of bioaccumulation, including bioconcentration factors, bioaccumulation factors, biomagnification factors (BMFs), biota-sediment accumulation factors (BSAFs), and trophic magnification factors (TMFs). Thus, the accuracy of these metrics is determined by the error associated with measurement of lipid content. In order to reduce error and increase the accuracy of these metrics, measurements of lipid content must be made with a greater deal of certainty than is currently in practice. To demonstrate the importance of this point, the lipid content of whole-body homogenates of aquatic organisms (zooplankton, macro invertebrates, and fish) collected from a temperate freshwater lake were determined using two different methods. The mean percent difference between whole-body lipid content measured using the two methods was approximately 28% across all organisms (n=52). Lipid contents measured by the two methods were used to normalize residue concentrations for select polychlorinated biphenyl (PCB) congeners measured in the same whole-body homogenates. Both sets of lipid-normalized residue concentrations were used to determine BMFs, BSAFs, and TMFs for select PCB congeners. This presentation will discuss and expand upon the impacts that lipid content may have on these bioaccumulation metrics. This work was supported in part by the Centre Europeen des Silicones.

**WP174 In Vitro Metabolism Assessment of Several Fragrances using Human and Rainbow Trout Cryopreserved Hepatocytes** J.A. Weeks, S.C. Johnson & Son, Inc. / Product Safety, Toxicology & Enviro, S.C. Johnson & Son, Inc. / Global Environmental Safety; P.D. Guiney, S.C. Johnson & Son, Inc. / Global Safety, Regulatory & Environmental Assessme; K.M. Johanning, KJohanning Consultancy / dba of Pura Vida Connections, LLC; U. Doshi, A.P. Li, In Vitro ADMET Laboratories LLC. Limited information is available on the metabolism of chemicals other than drugs using *in vitro* methodology, in particular utilizing human and fish hepatocytes. Human hepatocytes are considered the gold standard in the metabolism of pharmacological compounds. Bioaccumulation assessment of test chemicals is required by REACH. Traditional *in vivo* methodologies using large numbers of animals with costly and labor intensive procedures have been used to determine BCF. *In vitro* systems are increasing little by little as alternative

methods that may substitute for *in vivo* BCF assessment reducing cost, numbers of animals, and time for the experiments. Metabolic rate can be a controlling factor for bioaccumulation estimation and of great importance since these will determine the fate of the parent compound. The main objective of this study was to determine *in vitro* metabolic fate of fragrance chemicals in human and trout cryopreserved hepatocytes. Nine fragrances (Galaxolide, Celestolide, p-Tolyl Acetate, Ambreton, Cashmeran, Traseolide, Tonalide, Ethylene Brassylate, and Pentadecanolide) were incubated in human and cryopreserved hepatocytes trout (male and female) and metabolic rates were compared. Preliminary incubations were done for all fragrances to determine the time necessary for the final metabolism determinations. GC-MS was utilized to analyzed the fragrances and determine loss of parent compound for metabolic rate calculations. Total cell density was  $0.5 \times 10^6$  cells/mL and fragrance concentration was 1  $\mu$ M. Fragrances were grouped in three categories according to overall turnover as: slowly (Galaxolide, Celestolide and p-Tolyl Acetate), moderately (Ambreton, Cashmeran, Traseolide, and Tonalide) and rapidly (Ethylene Brassylate and Pentadecanolide) metabolized. Differences in metabolic rates were found between human and trout cryopreserved hepatocytes. The results indicate that human cryopreserved hepatocytes metabolized Ambreton, Tonalide, Cashmeran, Pentadecanolide and p-Tolyl Acetate at a faster rate when compared with trout hepatocytes. Unlike these, trout cryopreserved hepatocytes metabolized Ethylene Brassylate and Traseolide at a faster rate than human hepatocytes. Galoxolide and Celestolide were metabolized by both human and trout cryopreserved hepatocytes at a similar rate. Cryopreserved hepatocytes as well as trout liver S9 incubations (performed previously with other fragrances) can be powerful tools in estimating BCF.

**WP175 Linking Bioavailability, Toxicity and Tissue Residue level in a Sediment Toxicity Identification Evaluation** D. Farrar, US Army Engineer Research and Development Center, US Army Engineer Research and Development Center / Environmental Laboratory; G. Lotufo, J.G. Sims, U.S. Army Engineer Research and Development Center / Environmental Laboratory. Under current approaches, sediment toxicity and bioaccumulation of contaminants are the major sediment quality characterization component for management decision making. Sediment toxicity Identification evaluations (TIE) have been developed for identifying compounds responsible for observed toxicity in sediments containing a mix of contaminants. Management of contaminated sediments would benefit from lines of evidence that identify chemicals that are bioavailable, result in toxicity, and are taken up into receptors of concern. One major limitation of our existing testing and evaluation is that measures of bioavailability, toxicity and bioaccumulation are not adequately linked to confidently establish a cause-effect relationship. Because most sediment remediation projects are driven by the presence of bioaccumulative compounds, understanding the influence of TIE amendments on tissue residue levels is extremely important. A 21-d *Leptocheirus plumulosus* phase I sediment TIE was conducted using a primarily PAH contaminated field collected sediment. Amendments were added to the test sediments to identify organic (activated charcoal) and metal (SIR-300 resin) induced toxicity. A mixture of charcoal and resin was also included for contaminant mixture interactions. Four concentrations of PAH contaminated sediment were utilized in order to establish a tissue residue-effects level for PAHs and metals. Tissue samples were collected to demonstrate uptake or absence of uptake in the presence of a contaminant and pore water TAL metals and PAH concentrations were measured as an indication of contaminant bioavailability. The activated carbon amendment provided the greatest reduction in PAH pore water concentrations, PAH tissue concentrations and observed toxicity. While pore water metal concentrations in the un-amended sediment were generally low, a reduction in toxicity was also observed with the SIR-300 amendment. The mechanism of the reduced toxicity with SIR-300 resin is unclear. Although pore water PAH concentrations were reduced by AC, tissue residue levels did not decrease by a corresponding proportion, suggesting another exposure route other than pore water, such as ingestion fine sediment particles, are major sources of contaminant uptake in *Leptocheirus plumulosus*.

**WP176 Modeling the bioaccumulation of polybrominated diphenyl ethers (PBDES) in the marine food web of Puget Sound, Washington, USA** P. Luk, Simon Fraser University / Department of Biological Sciences; J. Alava, Simon Fraser University / School of Resource & Environmental Management; G. Pelletier, Washington State Department of Ecology; F.A. Gobas, Simon Fraser University / School of Resource and Environmental

Management (Faculty of Environment), Simon Fraser University / School of Resource & Environmental Management, Faculty of Environment, Simon Fraser University. Polybrominated diphenyl ethers (PBDEs), a group of brominated flame retardants listed as persistent organic pollutants, has been identified in the North American aquatic environment. This group of chemicals is known to have endocrine disrupting effects and may readily bioaccumulate in aquatic foodwebs. The objectives of the study were to apply Biota-Sediment Accumulation Factors (BSAFs) and develop a foodweb bioaccumulation model for the Puget Sound aquatic environment that may be used to predict concentrations of PBDEs in organisms and derived sediment quality guidelines. The southern resident killer whale's food web of Puget Sound was used in the model. The rationale to use the food web of killer whales is based on the fact that these marine mammals are particularly vulnerable to heavy contamination by persistent organic pollutants, including PBDEs, and due to their high trophic level, long lifespan and high lipid content in blubber. The model makes use of empirical water and sediment concentrations of PBDEs, physical-chemical properties for PBDE congeners, biological parameters of aquatic organisms, diet composition and trophic interaction of the foodweb, uptake and elimination rates, and metabolic processes of PBDEs in organisms. These were integrated through the use of steady state mass balance equations. Although data for PBDE metabolic rates are limited and were unavailable for the current model application, these compounds, however, are able to be biotransformed by aquatic organisms. Thus, characterization of the biota PBDE concentrations incorporating metabolic processes may improve the modeling work and assessment of the potential adverse effects of PBDEs on the ecosystem. The predicted BSAFs determined by the model were compared with the observed BSAFs calculated from empirical data to assess the model's applicability and develop future sediment criteria targets.

**WP177 PAHs, Nitro-PAHs, Hopanes and Steranes in Lake Trout from Lake Michigan** L. Huang, S. Batterman, S. Chernyak, University of Michigan. This study characterizes polycyclic aromatic hydrocarbons (PAHs), nitro-PAHs (NPAHs), steranes and hopanes in lake trout from Lake Michigan. Many of these semivolatile organic compounds (SVOCs) are environmentally significant due to their persistence, bioaccumulation and toxicity; however, their distribution in top predator freshwater fish such as lake trout is largely unknown. SVOC measurements were made in male whole fish, female somatic tissues, and eggs collected at three offshore sites near Illinois, Michigan and Wisconsin. Concentrations of the SVOC groups differed by location and sample type, e.g.,  $\Sigma$ NPAH levels were lowest at the Illinois site (average of  $0.62 \pm 0.35$  pg/g), while eggs from the Wisconsin site had the highest levels ( $68 \pm 42$  pg/g). In contrast, sterane and hopane levels were lowest at the Michigan site ( $265 \pm 72$  and  $114 \pm 5$  pg/g, respectively), and NPAH compositions differed by site. Female somatic tissues had higher PAH concentrations than eggs, but NPAHs showed the opposite trend. These results show significant differences in exposure sources and metabolic fates of these SVOC groups.  $\Sigma$ PAH levels in the lake trout (0.24 to 1.25 ng/g) were much lower than levels reported earlier in small prey fish (10 to 1000 ng/g), indicating little potential for PAH biomagnification. Benzo[a]pyrene toxic equivalents (TEQs) calculated for PAHs ranged from 62 to 113 pg/g, and were dominated by benzo[a]pyrene and dibenzo[a,h]anthracene. Although NPAH concentrations were much lower, their greater toxicity led to quite similar toxicities with NPAH TEQs ranging to 62 pg/g. 6-nitrochrysene was the dominant NPAH in this assessment. Both PAH and NPAH TEQs fell under the Canadian limit (1 to 4 ng/g B[a]P), suggesting little potential for carcinogenic risks. However, additional assessment of NPAHs is warranted for several reasons: the NPAH TEQ had similar magnitude as the PAH TEQ; TEQs may not reflect ecological effects; and our results may not be representative of levels and risks in other species or areas.

**WP178 Sediment bioaccumulation of PCBs in four functionally and phylogenetically different infaunal invertebrates** G. Lotufo, US Army Engineer Research and Development Center / Environmental Laboratory; A. Kennedy, J. Coleman, T. Bridges, U. S. Army ERDC. Benthic organisms vary in terms of their position within the sediment column (e.g., epibenthic vs. infaunal), their relationship with and exposure to sediment particles and porewater (e.g., tube building vs. free burrowing and soft bodied vs. exoskeleton vs. shell), and feeding behavior, all of which are expected to contribute to differences in exposure to contaminants. More importantly, these differences suggest that the validity of generalizations made in contaminant modeling up the food chain is questionable. To evaluate exposure variation

among benthic species with varying functional attributes, toxicokinetic experiments were conducted using PCB-contaminated sediment (34 mg/kg sum PCB congeners) from New Bedford Harbor, MA. Experimental species were *Leptocheirus plumulosus* (surface deposit feeder), *Neanthes arenaceodentata* (tube-building deposit feeder), *Eohaustorius estuarii*, *Yoldia limatula* (free-burrowing surface deposit feeders) and *Mercenaria mercenaria* (infaunal filter feeder). The organisms were exposed to sediment and sampled in triplicate after 2, 7, 14 and 28 days (1, 4, 7, and 14 days for amphipods). A second group of organisms were exposed to the sediment for 28 days then transferred to clean sediment and sampled in triplicate after 3, 7 and 21 days for direct determination of depuration rates. Inspection of temporal bioaccumulation trends reveals steady state within 7 days for *L. plumulosus*, *N. arenaceodentata*, 21 days for *M. mercenaria* and >28d for *Y. limatula*. However, longer time to approach steady-state was estimated using depuration rate kinetics (15 d for *L. plumulosus*, 25 d for *N. arenaceodentata*, 19 d for *Y. limatula* and >21 for *M. mercenaria*). Biota to sediment accumulation factors (BSAFs) determined at or near steady-state was highest for *L. plumulosus* (5.2), followed by *Y. limatula* (3.3) and lowest for *Neanthes arenaceodentata* (0.9) and *Mercenaria mercenaria* (0.3). Data for *E. estuarii* will be presented. This wide range in BSAFs indicates that, in addition to bioavailability, functional attributes need to be considered when predicting bioaccumulation from sediment in infaunal invertebrates.

**WP179 Perfluoroalkyl Contaminants (PFCs) in environmental matrices collected in the Antarctic Peninsula** J. Alava, Simon Fraser University / School of Resource and Environmental Management (Faculty of Environment), Fundacion Ecuatoriana para el Estudio de Mamíferos Marinos (FEMM) / FEMM Filial Playas, Simon Fraser University / School of Resource and Environmental Management, Faculty of Environment; M.G. Ikononou, Institute of Ocean Sciences / Department of Fisheries and Ocean; P. Calle Delgado, Escuela Superior Politécnica del Litoral / Facultad de Ingeniería Marítima y Ciencias del Mar (FIMCM), Escuela Superior Politécnica del Litoral / Department of Marine Engineering Biological, Ocean Sciences and Natural Resources; M. Riofrio, Instituto Antártico Ecuatoriano (INAE) / Departamento Técnico Científico; F.A. Gobas, Simon Fraser University / School of Resource and Environmental Management (Faculty of Environment), Simon Fraser University / School of Resource & Environmental Management, Faculty of Environment, Simon Fraser University. Antarctica is one of the last frontiers of the planet to investigate the environmental transport and accumulation of persistent organic pollutants (POPs). Perfluoroalkyl contaminants (PFCs) are a group of anthropogenic and industrial substances widely used and have recently been enlisted in the Stockholm Convention for POPs as they represent a significant risk to wildlife and humans due to their high biomagnification potential and toxicity risks, especially in food webs of the northern hemisphere and Arctic. Under the Antarctic Treaty, one of the priority actions is to assess and monitor POPs, including PFCs, in Antarctica. Although the assessment of PFCs in the Antarctic continent is scarce, questions linger about the long-range environmental transport of these substances to the southern hemisphere and the capacity of these substances to bioaccumulate in Antarctic food webs. As part of an ongoing scientific collaboration between the Ecuadorian Antarctic Institute and Simon Fraser University (Canada), environmental and biotic samples, including sediments, feathers and feces of seabirds (southern giant petrel, *Macronectes giganteus*; and, Gentoo penguin, *Pygoscelis papua*) and lichen, were collected in Peninsula Antarctica during the 2009 Antarctic expedition. PFC concentrations were analyzed by liquid chromatography tandem mass spectrometry (LC/MS/MS) at the Institute of Ocean Sciences (DFO, Sidney, Canada). Preliminary analysis revealed concentrations of perfluoroheptanoic acid (PFHpA) in all feather samples (mean = 3.0 ng/g ww; range = 2.2-4.0 ng/g) and in 74% of penguin feces (range = 1.46-25.2 ng/g). All lichen samples exhibited concentrations of perfluorohexanesulfonate (PFHxS), ranging 0.30-1.35 ng/g ww, while perfluorobutyric acid (PFBA) and perfluoro-n-pentanoic acid (PFPeA) were measured in 80 and 40% of the lichen samples, respectively. Perfluorotetradecanoic acid (PFTA), a chemical that can persist for decades in humans, was measured in 60% of sediment samples. PFHpA, PFBA and PFPeA are byproducts of stain/grease-proof coatings on food packaging, couches and carpets, and PFHxS was used in fire fighting foams and carpet treatments and phased out of consumer products by 3M in 2000 due to health risks. While atmospheric transport of PFCs may explain the ubiquitous nature of these contaminants in the Antarctic Peninsula, military bases and infrastructure of nations established there may also be contributing sources of PFCs in Antarctic ecosystems.

**WP180 Acute and Chronic Toxicity of Chemically Toxic and Benign Nanoparticles on *Ceriodaphnia dubia***

**A. Harmon**, US Army Engineer Research and Development Center / Environmental Laboratory; **A.J. Kennedy**, US Army Engineer Research and Development Center, CEERD-EPR; **J.D. Laird**, US Army Engineer Research & Development Center / SpecPro Incorporated, U.S. Army Engineer Research & Development Center / Environmental Laboratory; **A.J. Bednar**, US Army Engineer Research and Development Center / Environmental Laboratory; **C. Detzel**, NanoSafe. This study aims to evaluate the toxicity of *Ceriodaphnia dubia* to 20 and 100 nm nanosilver (nAg) and nanogold (nAu) in soft reconstituted water. Standard toxicity tests have shown that *C. dubia* are less sensitive to silver in chronic than acute testing, due to the particles binding to the food; therefore, reducing the bioavailability of silver. An altered testing procedure adding food 8 hours following water renewal alongside standard testing procedures was evaluated in both acute and chronic testing. This altered feeding ration allows for the uptake of nanoparticles by the organisms that may otherwise not be available. In acute and chronic testing, *C. dubia* were more than 3 orders of magnitude less sensitive to nAu than nAg. When comparing acute and chronic 20nm nAu tests, 100% mortality occurred in the highest concentration (75 mg/L) chronically with 60% survival acutely, suggesting different mechanisms of toxicity. Altered chronic testing of 20nm nAg resulted in greater sensitivity in the survival endpoint and decrease in reproduction compared to standard testing procedures. This supports that the addition of food decreases sensitivity to the organism, likely through both changes in bioavailability and organism rigor. Dynamic Light Scattering (DLS) indicated an increase in particle aggregation size, size increase was observed as concentrations decreased in nAu. Further studies are needed to determine if toxicity is occurring by different mechanisms when comparing acute-to-chronic ratios and testing of different aquatic organisms.

**WP181 Agglomeration and Dissolution of Silver, Titanium Dioxide, and Aluminum Oxide Nanoparticles in Artificial Biological Fluids**

**A.M. Mayo**, Badger Technical Services; **D.R. Johnson**, U.S. Army Engineer Research and Development Center / Environmental Laboratory, U.S. Army / CEERD-EP-R, US Army Engineer Research and Development Center / Environmental Laboratory, US Army Engineer Research & Development Center / Environmental Laboratory; **A.J. Bednar**, **J.A. Steevens**, US Army Engineer Research and Development Center / Environmental Laboratory. As nanoparticles move through various organ systems, it is important to characterize the physical state and fate of engineered nanoparticles (NPs) in biological fluids. New *in vitro* approaches are allowing researchers gain insight into biofluid components that may be responsible for NP biological fate (e.g., the presence of natural surfactants, like phosphatidyl-choline and taurocholate, in artificial alveolar fluid and intestinal fluid, respectively, may serve to shield nanoparticles from the high ionic strength of the artificial biofluids). Thus, the purpose of this study was to evaluate the agglomeration and dissolution of engineered nanoparticles (NPs) in artificial biofluids. A 24 h time-course evaluation of 1mg/L silver (Ag) NPs, titanium dioxide (TiO<sub>2</sub>) NPs, and aluminum oxide (Al<sub>2</sub>O<sub>3</sub>) NPs in artificial biological fluids was conducted using field flow fractionation-inductively coupled plasma-mass spectrometry (FFF-ICP-MS), dynamic light scattering (DLS), spectrophotometry, and dissolution methods. Citrate- and PVP-Ag (20 & 80 nm) in artificial alveolar fluid and intestinal fluid showed similar settling patterns, with absorbance levels decreasing only 10% from t=0 to t=24h. These values were  $\pm 5\%$  the absorbance values when particles were in water alone. In contrast, these same particles in artificial interstitial fluid and gastric fluid (pH 1.5) show a 40% decrease in absorbance at 24 h. Rapid agglomeration of AgNPs appears to be occurring, as indicated by the sudden formation of black particulates upon adding 20nm Citrate- and PVP-Ag to all artificial biofluids tested. Citrate- and PVP-Ag (20 nm) showed marked size increases over 24 h in artificial interstitial, intestinal, and gastric (pH 1.5) fluids. The decrease in surface area resulting from this agglomeration may also have an effect on Ag dissolution rates. Dissolution data from 20nm citrate-Ag and citrate-PVP, as well as AgNO<sub>3</sub>, in artificial gastric fluid, interstitial fluid, intestinal fluid, alveolar fluid, and cerebrospinal fluid, suggesting a loss of Ag<sup>+</sup>, when compared with Ag<sup>+</sup> concentrations in water. The high concentration of chloride ions in many artificial biofluids may lead to the rapid formation of AgCl during AgNP dissolution, as also seen by Rogers *et al.* 2012. The effect seems most dramatic when AgNPs and AgNO<sub>3</sub> are added to gastric fluid pH 1.5 and 3.0. Additional studies at physiologically relevant settings, (i.e., temperatures, CO<sub>2</sub>) will also be discussed.

**WP182 Effect of Nanosized CeO<sub>2</sub> Catalyst on Particulate and Gaseous Components of Diesel Emissions**

**G. Mainelis**, Dept Env Sciences, Rutgers University; **J. Zhang**, Div of Env Health, Keck School of Medicine, University of Southern California; **L. Calderon**, Dept Env Sciences, Rutgers University; **L. Zhang**, **P.J. Liroy**, Env and Occup Health Sciences Institute; **K. Chung**, National Heart and Lung Institute, Imperial College London; **J. Zhang**, Div of Env Health, Keck School of Medicine, University of Southern California. Nanosized CeO<sub>2</sub> fuel catalyst is increasingly being used to improve the diesel combustion efficiency. However, it is unknown what effects the addition of nano CeO<sub>2</sub> into diesel fuel has on physicochemical and toxicological properties of diesel exhaust and the resultant health effects. As a first step toward answering those questions, we characterized diesel exhaust emissions due to the combustion of diesel fuel with nanosized CeO<sub>2</sub> additive of different concentrations. Different amounts of Envirox™ CeO<sub>2</sub> additive (Energenics Ltd., UK) were mixed with regular ultra-low sulfur diesel fuel to produce the following CeO<sub>2</sub> concentrations in the fuel: 0 ppm (diesel fuel only, or 0X of CeO<sub>2</sub>), 5 ppm (0.1X), 50 ppm (1X – manufacturer's recommendation), and 500 ppm (10X). We used a one-cylinder electrical generator (Yanmar) under 100% load and a portion of the exhaust was directed into a 25 m<sup>3</sup> stainless steel testing chamber where the exhaust mass concentration during characterization was maintained at approximately 300 µg/m<sup>3</sup>. A Scanning Mobility Particle Sizer and an Aerodynamic Particle Sizer were used to measure exhaust aerosol size distribution. Gas monitors were used to analyze concentrations of CO, CO<sub>2</sub>, and NO<sub>x</sub>. We observed that the total exhaust particle number concentration increased with increasing CeO<sub>2</sub> concentration in the fuel: from  $1.5 \times 10^5$  cm<sup>-3</sup> for diesel only (0X) to  $3.6 \times 10^5$  cm<sup>-3</sup> for 10X CeO<sub>2</sub>. Concurrently, the mean particle diameter by number decreased with increasing CeO<sub>2</sub> concentration: from approximately 160 nm for diesel only (0X) to 100 nm for 10X CeO<sub>2</sub> and the decrease was statistically significant. At the same time, CO<sub>2</sub> concentration increased with increase from 0X to 10X of CeO<sub>2</sub>: from approximately 1300 ppm to approximately 2250 ppm. Increasing CeO<sub>2</sub> concentration in the fuel also increased concentrations of NO<sub>x</sub> (from 4500 ppb to 10200ppb) and CO (from ~4 to ~6.4 ppm) in the exhaust. The results show that on a per exhaust particle mass basis, the use of CeO<sub>2</sub> additive led to a reduced size of exhaust particles, but might have resulted in an increased concentration of NO<sub>x</sub>. We are currently performing measurements of the total mass emissions at different CeO<sub>2</sub> concentrations. The data will be used to determine total particle and gas exhaust amounts at different concentrations of nanosized CeO<sub>2</sub> additive.

**WP183 Effects of aqueous metal nanoparticle exposures on RBL-2H3 cell viability and degranulation**

**V.A. Ortega**, University of Alberta / Department of Biological Science; **J.D. Ede**, **B. Montgomery**, **J.L. Stafford**, **G.G. Goss**, University of Alberta / Department of Biological Sciences. The emergence of nanomaterials has promised a revolution of the medical treatment, consumer product and technological fields, but has also raised concerns over the potential for their unique properties to adversely affect biological systems. Numerous cytotoxic studies have reported effects on cellular stress and apoptotic pathways, however there still remains a paucity of information on the potential for NPs to affect immune cell viability and function. In this study, we examined the potential for NPs to impact the viability of a rat basophilic leukemia (RBL) immune cell-line, and to modulate their cellular functions. RBLs are representative granulocytes that release intracellular granules to combat foreign antigens. This Fcε receptor-mediated process is known as degranulation and is part of the innate immune response. Since NPs can differ in their physico-chemical properties, their behavioural characteristics in solutions are typically assessed together with toxicity measurements. Thus, dynamic light scattering was used to characterize three aqueous metal-oxide polyacrylic acid encapsulated NPs (TiO<sub>2</sub>, CeO<sub>2</sub>, nanocapsule, void of a metal-oxide core) in cell media at various exposure concentration and time points. Results indicated some agglomeration occurring that increased their hydrodynamic size from their reported 5-9nm, but was not different between concentrations. Immunotoxicity was measured by exposing RBLs to NP concentrations up to 200mg/L over 48h and were analyzed for cellular viability and proliferation using flow cytometry and propidium iodide as a fluorescent marker for cell death. Results showed dose-dependent decreases in viability with increasing NP concentrations and exposure times. To examine the influence of NPs on RBL degranulation we dosed RBLs at both sub-lethal and lethal concentrations and showed that degranulation was inhibited at all doses including those that did not decrease cell viability. Degranulation was also fully abrogated when the IgE



antibody, used to sensitize RBLs for degranulation, was also exposed to NPs. Antibody binding affinity assays showed decreased Fcε receptor binding when exposed to NPs, which may provide a mechanism for decreased NP inhibitory effects on degranulation. Fluorescent microscopy was used to supplement the results of both cell and IgE NP exposure experiments in order to demonstrate the role NPs have on influencing Fcε receptor and IgE-mediated degranulation.

**WP184 Effects of TiO<sub>2</sub> nanoparticles on various cellular biomarkers in eastern oysters, *Crassostrea virginica*** B. Johnson, University of North Carolina Charlotte / Department of Biology, UNC Charlotte / Biology department; M. McCarthy, University of North Carolina Charlotte; D. Carroll, Wake Forest University; A.H. Ringwood, University of North Carolina Charlotte. The continued manufacturing and use of engineered nanoparticles makes it increasingly important to examine their effects on marine organisms. The purpose of these ongoing studies is to characterize the potential toxicity of TiO<sub>2</sub> nanoparticle preparations on eastern oysters, *Crassostrea virginica*. Studies were conducted with whole oyster and isolated tissue exposures to TiO<sub>2</sub> over a range of concentrations in natural sunlight as well as dark conditions. Cellular biomarkers (such as GSH, lipid peroxidation (LPx), and lysosomal membrane damage) were then evaluated in gill and hepatopancreas tissues. There were no effects on lysosomal destabilization levels of hepatopancreas tissues or LPx levels of gill tissues under either light or dark exposure conditions. However there was an increase in LPx levels in oyster hepatopancreatic tissues exposed to TiO<sub>2</sub> nanoparticles in combination with sunlight, suggesting photo-enhanced toxicity. These types of basic studies are essential for addressing the potential impacts of nano-engineered particles on fundamental cellular processes as well as marine organisms.

**WP185 Effects of water quality parameters on the fate and toxicity of silver nanoparticles to deposit feeder *Lumbriculus variegatus*** J. Son, J. Vavra, University of Nebraska-Lincoln / School of Biological Sciences; Y. Li, M. Seymour, University of Nebraska-Lincoln / Department of Civil Engineering; V. Forbes, University of Nebraska-Lincoln / School of Biological Sciences. Accelerated production and widespread use of silver nanoparticles (NPs) in various industries have raised issues concerning their potential release into the environment and subsequent impacts on ecological systems. Though still limited, existing results indicate that the fate and toxicity of silver NPs are influenced by a variety of environmental factors, which may interact in ways that are not yet fully understood. Given the potential variety and complexity of these interactions, it is imperative to increase our understanding of how environmental factors affect the fate and toxicity of silver NPs under a range of environmentally realistic conditions. We used a central composite circumscribed design (CCCD) to systematically explore how different realistic combinations of pH, hardness, and natural organic matter affect the surface charge (zeta potential), particle size distribution, and aggregation state of Ag NPs. The results of the CCCD study were used to predict a realistic worst-case exposure scenario for the oligochaete, *Lumbriculus variegatus*. Bioavailability and toxicity of Ag NPs were subsequently tested under this exposure scenario using a modified OECD 225 test design. The results of this study will improve our understanding of the factors affecting silver NPs in the environment and could ultimately be used to generate modeling approaches to extrapolate across different environmental conditions.

**WP186 Evaluating the toxicity of nano-silver hair dryers via physico-chemical characterization methods and microbiological assays** A.A. Taylor, S.L. Walker, University of California, Riverside. Nanotechnology infused personal care products are on the rise. Specifically, commercial hairdryers are marketed as emitting silver nanoparticles to give hair a sleek, shiny look while also promoting an antimicrobial effect. This study investigates the risks associated with the silver nanoparticles in regards to skin flora toxicity, inhalation exposure, and the fate of silver nanoparticles in the environment after removal from the hair. Alteration of the normal skin flora due to antimicrobial effects of silver nanoparticles can cause opportunistic pathogens to have an amplified chance to colonize the skin and cause harm to the consumer. Three brands of hairdryers were selected (price range from \$20-90) and using a micro-orifice uniform deposition impactor (MOUDI), particle samples from the hairdryers were collected based on size fractionation (0.056 – 18 μm). Scanning electron microscopy and energy-dispersive x-ray spectroscopy were performed for elemental analysis of the MOUDI particle samples for silver nanoparticle detection. Initial results demonstrate

that silver is not being emitted from the hairdryers. Following the characterization experiments, detecting a toxicity dose response relationship with the Kirby-Bauer assay over a range of silver concentrations will be performed with *Escherichia coli*, and *Staphylococcus aureus*, a common skin organism. Additionally, a survey was conducted at six retail stores for consumer hair dryer trends; it appears that silver nanoparticle hair dryers are only sold online and are not currently available in retail stores. This may help curb environmental exposures until nanoparticle hair dryers become more prevalent. Findings from this study will help consumers make more informed decisions based on the risks associated with new technologies.

**WP187 Gaining a critical mass: considering alternative nanomaterial dose metrics and mitigating factors for whole organisms** A.J. Kennedy, US Army Engineer Research and Development Center, CEERD-EPR; M. Hull, NanoSafe; P. Vikesland, Virginia Tech; M. Chappell, F. Hill, J. Laird, J. Stanley, A. Poda, A. Bednar, J. Steevens, ERDC. If engineered nanoparticles contaminate environmental media at toxicologically relevant levels, risk assessors will require an easy to implement dosimetry standard that is predictive of effects on traditional, population level effect endpoints. Increasing evidence suggests the mass-only toxicity paradigm inadequately standardizes the implications of nanoparticles to eco-receptors. While we continue to rely on mass measurement, since direct analytical determination of particle properties at low concentration is less convenient or unavailable, mass-based conversions to other dose metrics should be considered in the interim. In this presentation, we review several dose metric calculations for nanosilver such as particle size, number, total dispersion surface area, particle number density and the dissolved fraction. Mass-based dosimetry resulted in divergent lethal median concentrations, indicating size dependent toxicity of nanosilver (20 – 100 nm) to *Ceriodaphnia dubia* and *Pimephales promelas*. Total surface area, however, better standardized observed toxicity. Examination of dose metric efficacy with increased environmental complexity suggested the total surface area metric was confounded by various factors (particle polydispersity, dissolved organic matter, UV light, ionic strength), resulting in under- or over-prediction of toxicity, depending on the factor. Nanosilver is characteristically much less toxic in environmentally relevant conditions compared to pristine laboratory media. Thus, just as water quality criteria for dissolved metals are scaled to water hardness, nanoparticle toxicity thresholds need adjustment for mitigating factors. Further, computational chemistry predicts temporal instability of citrate functional groups on nanosilver, resulting in oxidation and ion release. Empirical data indicating that the dissolved fraction and toxicity of nanosilver suspensions increases over time supports this prediction. Thus, in some cases total surface area dose metrics may co-vary with direct causes of toxicity, such as ion release from dispersions of smaller (but higher total surface area) nanosilver particles. Relating novel dose metrics to nanosilver toxicity may be impractical for dispersions with high dissolved fractions in which ions will dominate toxicity, as confirmed by similar mass-based LC50 values between particle suspensions and supernatants of the same suspension.

**WP188 Hazard identification and bio-distribution analysis of sub-nanosilver particles following dermal administration** T. YOSHIDA, National Institute of Biomedical Innovation (NiBio) / Laboratory of Biopharmaceutical Research (LBR), Graduate School of Pharmaceutical Sciences, Osaka University / Laboratory of Toxicology and Safety Science; Y. Yoshioka, T. Hirai, M. Uji, K. Ichihashi, K. Misato, H. Takahashi, A. Uda, T. Mori, Graduate School of Pharmaceutical Sciences, Osaka University / Laboratory of Toxicology and Safety Science; S. Tsunoda, National Institute of Biomedical Innovation / Laboratory of Biopharmaceutical Research; H. Nabeshi, National Institute of Health Science; T. Yoshikawa, Y. Tsutsumi, Graduate School of Pharmaceutical Sciences, Osaka University / Laboratory of Toxicology and Safety Science. With development of nanotechnology, nanomaterials (NM; ~100 nm) and sub-nanomaterials (sNM; ~10 nm) has been used widely in various applications. For example, silver particles show strong anti-bacterial activity, so they have been widely used in foods and cosmetics as an anti-bacterial agent. Thus, the exposure against NM and sNM is unavoidable for us in our living environment. In most cases, however, the safety evaluation of NM and sNM has been insufficient for ensuring their safety. Therefore, it is necessary to analyze hazard and bio-distribution of NM and sNM. Here, we examined the biological effect and bio-distribution of silver particle following dermal exposure. In this study, we used nanosilver particle (nAg; particle size is 20 nm), sub-nanosilver particle (snAg; particle size is 1 nm) and silver nitrate aqueous solution (as

Ag ion control). We treated to BALB/c mice with 0.1 mg /head by single dermal administration on the both ears for 7 days. After 24 hours of the last administration, we performed hematology analysis and pathological examination. As a result, there were little differences about all the parameter of haematology analysis among all groups. On the other hand, in pathological examination, the abnormal pathological findings were observed in both ears only in snAg-treated group. Next, we examined the bio-distribution of nAg and snAg by inductively-coupled plasma mass spectrometry (ICP-MS) analysis. ICP-MS analysis showed that only snAg penetrated skin and accumulated some tissues, such as liver or spleen, after topical application. On the other hand, nAg accumulated in the dermal administration site and was not detected in the any tissues. Collectively, we revealed that snAg might show different biological effect and absorption compared to nAg and solution of silver nitrate. Now, to accumulate safety information about snAg following dermal administration, we are trying to collect ADME (absorption, distribution, metabolism and excretion) information. We believe that our study will provide helpful information for safe use of NM and sNM in the future.

**WP189 How water quality parameters affect the surface charge and aggregation of copper oxide (CuO) nanoparticles** J. Son, J. Vavra, University of Nebraska-Lincoln / School of Biological Sciences; Y. Li, M. Seymour, University of Nebraska-Lincoln / Department of Civil Engineering; V. Forbes, University of Nebraska-Lincoln / School of Biological Sciences. With the rapid development of nanotechnology and its applications, it is inevitable that nanoparticles (NPs) are released into the environment, where their fate and behavior are largely unknown. Moreover, it has been recognized that individual environmental factors could play an important role in the fate of metal NPs, which in turn could influence their toxicity to ecological receptors. NPs that enter natural aquatic systems will be exposed to a variety of environmental factors which may interact in complex and unexpected ways. In this study, we used a second order central circumscribed design to systematically investigate how water quality parameters (pH, hardness and natural organic matter) influence the surface charge (zeta potential), particle size distribution, and partitioning of copper oxide (CuO) NPs in OECD reconstituted water. The results of this study will contribute to quantifying the interactions of CuO NPs with water quality parameters; in particular, the results will play a significant role in understanding under which environmental condition CuO NPs are likely to become problematic. This knowledge can ensure that realistic worst-case exposure scenarios are considered in the toxicity testing and risk assessment of CuO NPs.

**WP190 In Vitro Co-culture System to Examine the Effects of Inhaled Nanoparticles** K.B. Donohue, U.S. Army Engineer Research and Development Center / Environmental Laboratory, US Army Corps of Engineers / Environmental Laboratory; A.M. Mayo, Badger Technical Services; D.R. Johnson, US Army Corps of Engineers / Environmental Laboratory; J.A. Steevens, U.S. Army Engineer Research and Development Center. Engineered nanoparticles (NPs), with at least one dimension in the 1-100nm range, possess numerous potential benefits to society in fields as diverse as electronics, textiles, medicine, energy and construction. The respiratory system represents a unique target for the potential toxicity of NPs. Due to their dimensions, inhaled NPs can reasonably be expected to penetrate to the deepest part of the lungs, the alveolar sacs. Completed inhalation studies in laboratory rats have demonstrated that some NPs induce oxidative stress, inflammation and fibrosis. In this study, we examine the effects of 4 different types of NP exposure (nano-silver [nAg] with citrate or polyvinylpyrrolidone [PVP] coating, nano-titanium [nTiO<sub>2</sub>] and nano-bismuth [nBi]) on an alveolar co-culture model consisting of human type II pneumocytes and alveolar macrophages. Co-cultures were set up with a ratio of 3:1 of type II pneumocytes to each alveolar macrophage 24 hours prior to treatment. Media was then removed and co-cultures were treated with varying concentrations of NPs for 24 hours. The effects of NP exposure on the co-cultured cells were then examined by cell viability, cell phagocytosis and cytokine secretion. The viability of the cells after 24 hours of treatment was determined by propidium iodide staining. Cell phagocytosis was measured by a phagocytosis assay kit. The number of cytokine-secreting cells was determined by Intracellular Cytokine Staining (ICS) while the concentration of cytokines was determined by ELISA using media collected from the treated cultures at 24 hours. NP exposures resulted in a slight decrease in cell viability at the highest doses only. Citrate-nAg and PVP-nAg increased

the percentage of cells secreting the inflammatory cytokines interleukin-1beta (IL-1β) and tumor necrosis factor-alpha (TNF-α). In contrast, nTiO<sub>2</sub> exposure decreased the percentage of cells secreting IL-1β and TNF-α. However, no change was seen in the percentage of cells secreting the immunosuppressive cytokine transforming growth factor-beta (TGF-β) in response to nAg or nTiO<sub>2</sub> exposures. The increase in inflammatory cytokine secretion agrees with the rat *in vivo* data, suggesting that this *in vitro* alveolar co-culture model may adequately predict the inhalation toxicity of NPs.

**WP191 In vivo study of the role of endocytosis in silver nanoparticle uptake, localization and toxicity in *Caenorhabditis elegans*** X. Yang, / PHD student; A.R. Badireddy, C. Jiang, Duke University / Civil and Environmental Engineering; H. Hsu-Kim, Duke University; D.E. Hinton, Duke University / Nicholas School of the Environment; M. Wiesner, Duke University / Civil and Environmental Engineering; J.N. Meyer, Duke University / Nicholas School of the Environment. We and others have observed that silver nanoparticles can be incorporated into the tissues of metazoans, but the mechanism of uptake is not well understood. In our study, nematode *Caenorhabditis elegans* was used as a model organism to study the role of endocytosis in Ag NP uptake, Ag NP-mediated innate immune response and expected toxicity of Ag NPs. We used three endocytosis-deficient mutants (*rme-1*, *rme-6* and *rme-8*) and one innate immune-deficient mutant (*xbp-1*). Because we wanted to focus on the NP uptake and effects, we used citrate-coated Ag NPs (CIT<sub>7</sub>), which dissolve the least of all the Ag NPs we have tested. We found that the endocytosis-deficient mutants were more resistant to CIT<sub>7</sub> as compared to wildtype, while preliminary results suggest that *xbp-1* is more susceptible. None of those mutants showed altered sensitivity to ionic silver. In addition, we used the clathrin-mediated endocytosis inhibitor phenothiazine, which protected the nematodes from CIT<sub>7</sub> toxicity. These preliminary results indicate that clathrin-mediated endocytosis is required for CIT<sub>7</sub> toxicity, while suggesting that the innate immune response is protective against CIT<sub>7</sub> toxicity.

**WP192 Photo-enhanced toxicity of nano-titanium dioxide (anatase) on freshwater zooplankton** M. Alloy, University of North Texas / Department of Biological Sciences; A. Roberts, University of North Texas. Some engineered nanoparticles, including titanium dioxide have the potential to exert effects through photo-enhanced toxicity, similar to that associated with polycyclic aromatic hydrocarbons. Titanium dioxide has been used for decades as a paint pigment, food coloring, sun screen, the active portion of self-cleaning surfaces, fog free mirrors, and in many more roles prior to the use of nano-scale TiO<sub>2</sub> crystals. The first reports of TiO<sub>2</sub> photocatalytically decomposing compounds in the aqueous phase by generating reactive oxygen species was reported in the late 1970s. Toxicology of nano TiO<sub>2</sub> is not new, but few studies have looked at aquatic toxicity of nano TiO<sub>2</sub> under exposure to UV light. The goals of this study were: (1) to determine if nano TiO<sub>2</sub> was more toxic to aquatic organisms in the presence of UV light and (2) if toxicity followed patterns of reciprocity as previously described with PAHs, in outdoor microcosm exposure systems. An eight hour single exposure study using *Daphnia magna* adults as model organisms was performed to establish differences in immobility between exposures and controls under natural UV radiation. Fluoranthene, a well studied PAH phototoxicant, was used as a reference toxicant. After eight hours of natural light the organisms were removed from light exposure and immobility of thoracic appendages coupled with a lack of response to light stimulus was assessed. A significant difference in the endpoint was found between full UV and partial UV treatments (Tukey post hoc p < 0.01) and among TiO<sub>2</sub> exposures (ANOVA F = 23.54 p < 0.01 Tukey post hoc). We report that anatase nano TiO<sub>2</sub> was phototoxic under ambient solar UV in concentrations in the parts per billion range.

**WP193 Preliminary evaluation of the bioaccumulation potential of CeO<sub>2</sub> nanoparticles in fish via aqueous and dietary exposure** K. Rehberger, University of Heidelberg; E.R. Salinas, BASF SE / Experimental Toxicology and Ecology; S. Zok, BASF SE. In the face of increasing applications for nanotechnology, the potential for nanoparticles to bioaccumulate continues to be a major knowledge gap. The aim of this project was to evaluate bioaccumulation and biomagnification of nano-cerium dioxide (CeO<sub>2</sub>, uncoated, mean particle size 28 nm) in fish via water and diet respectively. Common carp were exposed to treatments of nano-CeO<sub>2</sub> (1 & 100 mg/L) and micro-CeO<sub>2</sub> (100 mg/L, mean particle size 200 nm) dispersed in water containing 10 mg/L humic acid for 10 days. As a separate treatment, carp



were fed nano-CeO<sub>2</sub> at 68 mg/kg in diet also for 10 days. Fish samples were collected on 3 occasions (at day 1, 4, 10), and water samples every third day. All samples were immediately analyzed for Ce by ICP-MS. The uptake of Ce was evaluated in selected tissues (liver, gastrointestinal tract, gills, skin and file) and the carcass from dissected fish. The highest amounts of Ce were found in the gastrointestinal tract in both feeding and water exposures. In skin and gills considerably lower amounts of Ce were found. In internal tissues such as liver and file measured Ce was generally below the quantification limit, with a few exceptions that were most likely due to contamination during dissection. The results indicate that there is a very low bioaccumulation potential for CeO<sub>2</sub> (BCF < 5 and BMF < 0.002). There was no difference in accumulation between nano- and micro-CeO<sub>2</sub>.

**WP194 The effect of hardness and humic acid on the influx of silver after exposure to aqueous and dietary silver nanoparticles in a freshwater snail**

T. Stoiber, University of California-Davis / John Muir Institute of the Environment; A. Lopez Serrano Oliver, University Complutense de Madrid / Department of Analytical Chemistry; M. Croteau, U.S. Geological Survey / Water Resources Discipline; S. Luoma, University of California-Davis / John Muir Institute of the Environment. We investigated the bioavailability of waterborne and diet borne silver nanoparticles (AgNP) coated with polyvinylpyrrolidone (PVP) and thiolated polyethylene glycol (PEG-SH) in waters of different chemistry using the freshwater snail, *Lymnaea stagnalis*. Specifically, waterborne and diet borne Ag uptake rates were measured in short-term experiments in snails exposed to a range of Ag concentrations in water hardness that varied up to 100-fold and humic acid (Suwannee River) concentrations that varied by 20-fold. Upon 24h exposure to waterborne PVP and PEG-SH AgNPs, uptake was linear over a range encompassing most environmental concentrations. Silver influx from PVP and PEG-SH NPs was faster in deionized water (rate constants of uptake,  $k_{uw}$ , of  $2.2 \pm 0.02$  and  $3.1 \pm 0.7$  l g<sup>-1</sup> d<sup>-1</sup>, respectively) compared to that in moderately hard water (MOD;  $k_{uw}$  of  $0.7 \pm 0.1$  and  $0.5 \pm 0.02$  l g<sup>-1</sup> d<sup>-1</sup>, respectively), suggesting a possible effect of competing cations in harder water. Both PVP and PEG-SH capped Ag were more bioavailable than citrate capped Ag (Croteau et al, 2011) in MOD. Additionally, silver influx in the snails reached saturation at higher Ag concentrations in the water as hardness decreased. Particle coating greatly influenced the factors that determine translocation. Higher concentrations of humic acid reduced uptake rates about the same as hardness (2-fold) across a range of environmentally relevant concentrations (0.5-10 mg/l). All forms of AgNP's are bioavailable to food webs from water and diet. Uptake of Ag from AgNP's from water is affected similarly by coating-type and by water chemistry. Uptake into food webs from diet is affected by at least the type of coating on the AgNP.

**WP195 The Role of Surface Chemistry Toxicity of CeO<sub>2</sub> Nanoparticles to *Caenorhabditis elegans***

E.K Oostveen, University of Kentucky / Department of Plant and Soil Sciences, / Department of Plant & Soil Science; B. Collin, O. Tsyusko, University of Kentucky / Department of Plant and Soil Sciences; U. Graham, University of Kentucky / Center for Applied Energy Research; J. Unrine, University of Kentucky / Department of Plant and Soil Sciences. Manufactured ceria (CeO<sub>2</sub>) nanoparticles (NP) are valued for their ability to transition easily between +3 and +4 oxidation states, rendering them a useful catalyst in various applications. Surface coatings are usually applied to NPs, promoting stability and decreasing aggregation. The molecular weight and charge of surface coatings can potentially affect CeO<sub>2</sub>'s bioaccumulation and toxicity to soil dwelling organisms. We synthesized 4 nm CeO<sub>2</sub> coated with a neutral 10 kDa dextran (DEX) ( $\zeta$  potential = -0.731 mV in DI H<sub>2</sub>O, pH 6.88), positively charged diethylaminoethyl-dextran (DEAE) (+26.9 mV in DI H<sub>2</sub>O, pH 7.41) or negatively charged carboxymethyl-dextran (CM) (-29.3 mV in DI H<sub>2</sub>O, pH 6.19). The particles were characterized by transmission electron microscopy as well as dynamic light scattering and phase analysis light scattering (to determine  $\zeta$  potential). We exposed the model soil organism *Caenorhabditis elegans* to DEX-, DEAE-, and CM- coated CeO<sub>2</sub> in reconstituted moderately hard water. DEAE-CeO<sub>2</sub> was more lethal than both DEX- and CM-CeO<sub>2</sub>, likely due to positively charged particles interacting to a greater extent with cell membranes. We observed significant increases in mortality at DEAE-CeO<sub>2</sub> concentrations as low as 50 mg/L while no significant mortality was observed for the Dex-CeO<sub>2</sub> treatment at concentrations of up to 5000 mg/L. We observed no significant mortality for CM-CeO<sub>2</sub> particles up to 1000 mg/L. We concluded that positively charged surface coatings dramatically increase the toxicity of CeO<sub>2</sub> particles, while negatively charged or neutral

coatings decrease particle toxicity. Effects on *C. elegans* reproduction are currently under investigation.

**WP196 Toxicity testing of silver nanoparticles with benthic organism**

*Lumbriculus variegatus* J.V Kukkonen, University of Eastern Finland, Joensuu Campus / Department of Biology, University of Jyväskylä / Dept. Biological & Environmental Science; J. Rajala, University of Eastern Finland / Department of Biology; W. Qin, Nanjing University / School of the Environment; G. Waissi-Leinonen, K. Pakarinen, K. Maenpää, J. Akkanen, University of Eastern Finland / Department of Biology; L. Yang, Nanjing University / School of the Environment; J.J. Scott-Fordsmand, Aarhus University / Department of Terrestrial Ecology. The fate and effects of silver nanoparticles (AgNP) in the aquatic environment is under exploration and the possible risks that they may cause to aquatic organisms are still mostly unknown. It is likely that silver nanoparticles may pose a threat in aquatic systems through disruption of ion regulation and energy metabolism. It is also warranted to clarify the suitability of standard test methods to study the toxicity of silver nanoparticles for aquatic organisms. We aimed to examine toxicity of silver nanoparticles (AgNP) with freshwater benthic Oligochaeta worm *Lumbriculus variegatus* in two artificial and two natural sediments. Further, we investigated suitability of the OECD standard test methods for AgNP amendment into the sediments (OECD guideline nro. 222), and toxicity testing (OECD guideline nro. 225). Silver nitrate (AgNO<sub>3</sub>) was used as a reference compound in the toxicity tests. The standard test methods were found to be suitable for AgNP toxicity testing with sediment dwelling *L. variegatus*. The sediment spiking concentrations up to 1200 mg AgNP/kg sediment dw did not cause toxic effects while the similar exposure concentrations of AgNO<sub>3</sub> caused significant toxicity. The exception was one natural sediment where spiked AgNP significantly affected the feeding rate of *L. variegatus*. This study suggests that the acute toxicity of AgNP in sediments is low compared to ionic silver or that uptake of AgNP is slower and thus effects appearing later.

**WP197 Investigation of the Factors Responsible for the Ecotoxic Effects of Nano Titanium Dioxide on Aquatic Organisms**

A. Nakamura, The University of Tokyo / Graduate School of Frontier Science; S. Hirano, National Institute for Environmental Studies / Center for Environmental Risk Research; M. Yamamuro, The University of Tokyo / Graduate School of Frontier Science; N. Tatarazako, National Institute for Environmental Studies / Center for Environmental Risk Research. Manufactured nanomaterials are being utilized in various fields due to their superior characteristics, but at the same time, adverse effects on humans and wildlife have also been reported. Since the 2000s, plenty studies have been reported about the ecotoxic effects of industrial nanomaterials for aquatic organisms in terms of quantity. In recent years, the trend of this branch is shifting to more advanced approach such as combined effects with other elements or transitions and concentration in the food web. However, it essential question has not been considered sufficiently that what is the critical toxic factor. This is because existing researches did not grasp and control behavior of nanomaterials in water. It is an urgent issue to clarify the factors of impact in order to evaluate ecotoxic effects of nanomaterials for aquatic organisms property. Therefore, we investigated factors responsible for the ecotoxic effects of nanomaterials on aquatic organisms by sensitivity analysis approach using titanium dioxide that regarded chemically stable and non-toxic as a bulk material. First, dispersions were prepared that controlled their conditions in regularly. Target factors are as follows: dispersion concentration, particle size distribution, specific surface area, and particle number. Then, eggs of zebrafish were exposed to above dispersions in compliance with OECD guidelines for the testing of chemicals No.212: fish, short-term toxicity test on embryo and sac-fry stages. On the occasion of the exposure, light condition was also set intentionally. Before and during exposure, condition of titanium dioxide and its dispersions were measured by various methods such as dynamic light scattering method, scanning electron microscope, Brunauer-Emmett-Teller Method. After the exposure, effects of each case were analyzed from endpoints such as hatchability, survival rate, and etcetera. On it, change of degrees of effects accompanying with each factor were analyzed one by one. From the above relationships between the values of each element and degrees of effects were investigated.

**WP198 Transport and chemistry of silver nanoparticles in Wastewater Treatment Pilot Plant (Preliminary data)**

M. Belinga; R. Merrifield, University of Birmingham; J. Lead, University of Birmingham / School



of Geography, Earth and Environmental Scien. Understanding the fate, behaviour and transportations of silver nanoparticles (Ag-NPs) in wastewater treatment plant is of great importance in today's society due to concerns about potential human and environmental health. In this work, Ag-NPs were synthesised by reduction of silver nitrate with sodium borohydride and stabilised by citrate, polyvinylpyrrolidone (PVP) or by poly(ethylene glycol)-thiol (PEG-SH). Ag-NPs have the average core diameter of 10 nm. A suspension of 500 ppb Ag-NPs was dispersed in OECD synthetic sewage used as feed for the system and their behaviour was investigated. The surface plasmon resonance (SPR) spectrum showed a reduction of the peak height and a broadening of the peak at ca. 400 nm within 24 hours. With the atomic absorption spectroscopy (AAS), a decreasing of the particle concentration of 45%, 42% and 20% for citrate, PEG and PVP coated Ag-NPs respectively, was observed within 24 hours after their dispersion in the media. Ag-NPs were dispersed into a wastewater treatment pilot plant and their physical and chemical transportation monitored. A multi-method approach was used and the results will be discussed.

**WP199 Fate, transport, and toxicity of metal oxide nanomaterials in a soft-sediment estuarine system** S.K. Hanna, University of California; R.J. Miller, University of California Santa Barbara / Marine Science Institute; H.S. Lenihan, University of California Santa Barbara / Bren School of Environmental Science and Management. Engineered nanomaterials (ENMs) often possess unique physicochemical properties compared with their larger counterparts and are being incorporated into commercial and industrial products at an accelerating pace. Rising production and use of these products will lead to increased environmental release, exposure, and possible accumulation of ENMs in estuarine and marine sediments. We examined the fate, transport, and toxicity of three prevalent metal oxide ENMs, zinc oxide (ZnO), copper oxide (CuO), and nickel oxide (NiO) in estuarine microcosms containing amphipods, *Leptocheirus plumulosus*, as a means of estimating potential impacts of ENM exposure on an ecologically and toxicologically relevant species. Amphipods are important prey for a number of different species and are essential in sediment mixing in coastal environments. We exposed amphipods to concentrations of ENMs ranging from 100 to 2000 mg kg<sup>-1</sup> in sediment for 10 days. We determined amphipod mortality and collected surviving amphipods as well as water and sediment samples to determine the fate and transport of the ENMs. Mortality of amphipods increased with increasing ZnO and CuO ENM concentrations but we found no similar relationship for NiO ENMs. The LC<sub>50</sub> of ZnO and CuO ENMs were 770 and 870 mg L<sup>-1</sup> respectively, which is comparable with that of ionic Zn found in a previous study but higher than expected for Cu. Amphipods exposed to higher concentrations of ZnO, CuO, and NiO ENMs in sediment accumulated greater amounts of Zn, Cu, and Ni, but had higher concentrations of Cu than any other metal tested. Zn, Cu, and Ni concentrations in pore water and overlying water samples also increased with increasing ENM concentrations. We found up to nine times more Zn in water samples than Cu or Ni, suggesting that ZnO ENMs dissolve more rapidly than the others. We conclude that dissolution of ENMs is the main factor contributing to toxicity in *L. plumulosus* but that amphipods accumulate dissolved as well as particulate metals, suggesting possible exposure pathways to higher taxa.

**WP200 An interlaboratory study measuring sex steroids with RIAs and/or ELISAs: are we comparing apples to oranges?** A. Feswick, University of New Brunswick / Biology, University of New Brunswick; M. Fuzzen, University of Waterloo / Biology; K. Jensen, U.S. EPA / National Health and Environmental Effects Research Laboratory; K.J. Kroll, University of Florida / Physiological Sciences; A. Lister, Wilfrid Laurier University / Department of Biology, Wilfrid Laurier University; G.R. Tetreault, University of Waterloo / Biology; G.T. Ankley, US EPA / Office of Research and Development, National Health and Environmental Effects Research Laboratory, Mid-Continent Ecology Division, U.S. EPA / National Health and Environmental Effects Research Laboratory; N.D. Denslow, University of Florida / Department of Physiological Sciences and Center for Environmental and Human Toxicology, University of Florida / Dept. of Physiological Sciences &; M.E. McMaster, Environment Canada / National Water Research Institute; D. MacLatchy, Wilfrid Laurier University / Canadian Rivers Institute, Wilfrid Laurier University / Department of Biology; M.R. Servos, University of Waterloo / Department of Biology and Canadian Water Network, University of Waterloo / Department of Biology, University of Waterloo / Canadian Water Network; M.R. Van den Heuvel, Canadian Rivers Institute, University of

Prince Edward Island; K.R. Munkittrick, University of New Brunswick / Canadian Rivers Institute, University of New Brunswick / Department of Biology. Endocrine-disrupting chemicals (EDCs) are exogenous substances released into the environment that can lead to adverse reproductive effects in fish by a number of mechanisms including altering circulating levels of estradiol (E<sub>2</sub>), testosterone (T) and 11-ketotestosterone (11KT). Common methods to measure steroids in plasma samples include radioimmunoassays (RIAs) and enzyme-linked immunosorbent assays (ELISAs). This study had four primary objectives: (1) to compare precision and accuracy of RIA with ELISA, (2) to determine whether or not our ability to quantify plasma hormone concentrations has improved in over the past decade, (3) to determine the effect of using a standardized extraction on measurement variability and (4) to examine if the accuracy and variability of low and high hormone levels is constant across all concentrations (i.e. at either end of a standard curve). Wild white sucker (*Catostomus commersoni*) were collected from northern Lake Superior, near Terrace Bay, Ontario (48°50'N, 86°58'W), at either Little Gravel River (reference site) or Sawmill Creek (exposed site). At Sawmill Creek, white suckers are exposed to bleached kraft mill effluent. Preliminary results suggest that steroid values differ between RIA and ELISA methods, and caution should be used when comparing data from studies using differing methods. However, our ability to distinguish populations of fish that are exposed to EDCs such as pulp mill effluent is likely unaffected. Based on the results of this inter-laboratory study, we will provide recommendations to improve accuracy and precision of steroid measurements in fish ecotoxicology studies.

**WP201 Basic proteomics for toxicology using an inexpensive 2-D gel system** R. Sauterer, Jacksonville State University / Biology; S. Sparks, Jacksonville State University. 2 dimensional gel electrophoresis using IPG strips provides a useful initial evaluation of proteomic changes in organisms exposed to toxins or from contaminated waters. While most IEF systems using IPG strips are expensive, the Invitrogen Zoom IPG runner is an inexpensive alternative especially suitable for smaller institutions and student use. However, this system is limited to 2000 V, resulting in less IEF focusing and more horizontal streaking in the final 2-D gel than the more expensive systems that run at 10,000 volts. We investigated, using a standardized *E. coli* protein sample separated on 7 cm pH 4-7 IPG strips, a range of parameters in order to provide the best resolution and least horizontal streaking using this system. Gel resolution was relatively insensitive to sample load, with about 50 ug being optimal. Short runs of 1700 V-hr and runs at 4200 V-hr showed little difference, while runs of 11,000 V-hr or more resulted in decreased resolution. Either 0 or 0.2% ampholines added to the IEF sample gave better resolution with runs of 4200 V-hr or less, while longer runs required higher (0.5 – 2%) ampholines. pH 3-10 ampholines resulted in better resolution than mixes of 4-6 and 5-8 ampholines in all cases. Detergents in IEF sample buffers are critical to solubilize and denature proteins for optimal IEF focusing. The best resolution was obtained using a detergent mixture of ASB-14, SB-3-10 and CHAPS in 5M urea + 2M Thiourea in the sample buffer. SB 3-10 precipitated out in buffers of 7M urea + 2M Thiourea, resulting in poorer resolution. With single detergents, best resolution was obtained using 1% ASB-14 in 7M urea + 2M Thiourea, followed by 2% SB 3-10 in 7M urea + 2M Thiourea. Addition of SDS to IEF sample buffers resulted in little difference at less than 10% V/V ratios with the other detergents, while higher SDS concentrations caused massive streaking due to charging of proteins by the negatively charged SDS. Preliminary studies using animal tissues indicate that best resolution is obtained by homogenizing the tissue in an SDS-containing buffer, followed by cold 10% TCA-acetone precipitation of the proteins and solubilization in IEF sample buffer.

**WP202 Beneficial Use Impairment #4: Fish Tumors and Other Deformities – Use of Oxidative Stress Biomarkers as an Indicator of Impairment** D.S. Millsap, U.S. Fish and Wildlife Service; D.W. Sparks, U.S. Fish & Wildlife Service. Beneficial Use Impairment (BUI) #4, Fish Tumors and Other Deformities is one of the delisting criteria for the 43 Area of Concerns (AOCs) within in Great Lakes watersheds, relying on fish health assessments of brown bullhead catfish and white suckers to meet delisting criteria. Cyprinid and Ictalurid species from a PAH contaminated river in northern Indiana were assessed for external/morphological abnormalities and oxidative stress responses correlated with chronic PAH exposure. Catalase and superoxide dismutase activity decreased ( $p = 0.05$ ) in the cyprinids, goldfish (*Carassius auratus*) and bluntnose minnow (*Pimephales notatus*) and

in bullhead catfish (*Ameiurus* spp.). Glutathione peroxidase and glutathione reductase activities were also altered correlating with sediment PAH concentrations. Problems with applying BUI #4 in severely contaminated waters will be discussed.

**WP203 Brain acetylcholinesterase activity as diagnostic biomarker of water contamination in the guppy *Poecilia vivipara*** E.G. Gomes, Federal University of Rio Grande-FURG; A.A. De souza machado, Federal University of Rio Grande-FURG / Institute of Oceanography; A. Bianchini, Fundaco Universidade. Concentrations of metals, hydrocarbons and pesticides can potentially reach toxic levels in aquatic systems. In turn, biomarkers are meaningful tools to access the biological effects of these contaminants. Acetylcholinesterase (AChE) activity is a biomarker related to degradation of the neurotransmitter acetylcholine and enzyme activity changes are linked to behavioral disturbances. It has been pointed as being responsive to organic compounds exposure, but the specificity of its response to a suite of chemical contaminants is still not solved. Therefore, we analyzed the effect of the exposure (96 h) to copper (control, 5, 9 e 20 µg Cu/L), phenanthrene (control, 10, 20, 100 µg/L) and atrazine (control, 4, 10 and 100 µg/L) on brain AChE activity in the guppy *Poecilia vivipara* acclimated to salt water (salinity 24 ppt) at 20°C. For phenanthrene and atrazine we also assessed the effect of the solvents used to dissolve the contaminants (DMSO and methanol, respectively). Concentrations of copper were verified by AAS, while atrazine and phenanthrene concentrations were confirmed by GC. Brain AChE activity was determined by the conjugation of DNTB with acetylcholine iodate. Copper, phenanthrene and solvents did not affect brain AChE activity at the tested concentrations ( $p > 0.05$ ). However, 100 µg atrazine/L significantly increased brain AChE activity ( $p < 0.05$ ). Moreover, a significant and positive correlation was observed between AChE activity and waterborne atrazine concentration ( $r = 0.56$ ,  $p < 0.05$ ). These findings confirmed the specific AChE sensitivity to pesticides (atrazine) exposure. In fact, fast swimming erratic activity and other similar behavioral disturbances have been reported to be associated with atrazine exposure. In the other hand, copper has been described to affect AChE activity only at concentrations of mg/L. In fact, our data suggest that environmentally relevant copper concentrations will likely not affect AChE activity in *P. vivipara*. Regarding PAHs, some hydrocarbons have been shown to affect AChE activity. Our results show that phenanthrene is not affecting the AChE activity in *P. vivipara* at the concentrations tested. In summary, our findings indicate the brain AChE activity in *P. vivipara* as a suitable, specific and diagnostic biomarker for monitoring fish exposure to atrazine in salt water. [Support: Canadian IDRC and Brazilian CNPq (INCT-TA) and CAPES (Cincias do Mar)].

**WP204 Cytochrome P4501A as a Biomarker of Marine Environmental Contamination in the Scribbled Rabbitfish (*Siganus spinus*)** C. Emborski, Texas Tech University / Department of Environmental Toxicology; J.S. Biggs, University of Guam. Several classes of carcinogenic environmental organic pollutants, including polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and dioxins, negatively affect aquatic ecosystems worldwide. Pollutant detection is often difficult and expensive, especially when dealing with complex mixtures. Biological markers are informative tools to identify food sources that may harbor toxic compounds and areas unsuitable for recreation. Currently, there are no species, nor biomarkers, established for monitoring coral reefs in the Indo-Pacific region. This study evaluated the inducibility of the cytochrome P4501A (CYP1A) system in the scribbled rabbitfish, *Siganus spinus* (Siganidae), as a biomarker for organic pollutant exposures in coastal environments of the tropical Indo-Pacific. The following hypothesis was tested: Cytochrome P4501A (CYP1A) enzyme system expressed by *S. spinus* is dose- and time-dependently induced following exposure to the organic pollutant b-naphthoflavone (BNF). *S. spinus* hepatic CYP1A enzymatic activity and protein level rose as a function of dose during the first two days following a single intraperitoneal (IP) injection of BNF, and slowly returned to levels close to normal after 16 days, as measured using the 7-ethoxyresorufin-O-deethylase (EROD) and the non-competitive enzyme-linked immunosorbent (ELISA) assays, respectively. These findings support using the inducible CYP1A system of *S. spinus* as a biomarker for physiologically relevant coastal marine pollution exposures.

**WP205 Ecological Effects of Nutrient Pollution on Grand Bahama Island** C. Bowe, Environmental Monitoring & Risk Assessment Division. Islands are prevalent in tropical regions across the world. Most are part of developing countries and have scarce land based resources. Water resources

are especially limited on very small islands, many have no surface water resources and rely on limited groundwater resources in the form of freshwater lenses. The exposure of islands makes them particularly vulnerable to natural disasters such as floods and hurricanes and prolonged periods of drought. Pollution from population centres, industry and from agricultural and other activities is an increasing problem. The presence of industry and the disposal of nutrient raw material and waste provides a particular problem, eutrophication. Limited research data is available on island hydrology in The Bahamas and greater efforts are required to better understand the effects of human activities on the fragile water resources of the Atlantic archipelago of The Bahamas, particularly the industrialized island of Grand Bahama.

**WP206 High-throughput compatible bioassays for detecting endocrine disrupting chemicals in water supplies** K. Bi, G. Pelanek, Life Technologies Company. Public concern over health issues relating to endocrine disruptors and pharmaceutical in the water supply are mounting, particularly as related to the unknown long-term effects on infants and children. Sensitive bioassays that can assess the functional activity of an unknown compound for potential endocrine disruption would benefit routine testing of water supplies and allow establishment of limits that ensure public safety. Life Technologies has developed a broad portfolio of cellular bioassays that can be used to biological activity of key endocrine signaling pathways. These assays are simple, robust, reproducible and can be used in a high-throughput, automated systems. In addition, many have been applied to environmental toxicity testing and were selected for the TOX21 program initiated by National Toxicology Program (NTP), NIH and EPA. We will review the underlying technology for the bioassays and highlight their use for endocrine disruption assessment.

**WP207 Investigation of Adaptive Responses in Fathead Minnows (*Pimephales promelas*) Exposed to the Model Aromatase Inhibitor Fadrozole** K. Jensen, U.S. EPA / National Health and Environmental Effects Research Laboratory; J.E. Cavallin, E.J. Durhan, M.D. Kahl, E.A. Makynen, U.S. EPA; L.M. Thomas, US Fish and Wildlife Service / Mid-Continent Ecology Division; D.L. Villeneuve, U.S. EPA / National Health and Environmental Effects Research Laboratory, U.S. EPA; L.C. Wehmas, Oregon St. University / Mid-Continent Ecology Division; G.T. Ankley, US EPA / Office of Research and Development, National Health and Environmental Effects Research Laboratory, Mid-Continent Ecology Division, U.S. EPA / National Health and Environmental Effects Research Laboratory. The vertebrate hypothalamic-pituitary-gonadal (HPG) axis is a highly dynamic system, which, through various feedback mechanisms, strives to maintain physiological conditions conducive to reproduction even in potentially stressful situations. The development of useful predictive models of toxicity involves an understanding of how chemicals interact with the HPG axis temporally, in terms of both direct impacts and potential mechanisms for compensation during dosing, as well as recovery after cessation of chemical exposure. Fathead minnows (*Pimephales promelas*) were exposed to the aromatase inhibitor fadrozole (0.5 or 30 µg/L) either continuously for 1, 8, 12, 16, 20, 24, and 28 d or exposed for 8 d and then held in control water (no fadrozole) for an additional 4, 8, 12, 16, or 20 d and the time-course of effects on ovarian steroid production, circulating 17β-estradiol (E2) and vitellogenin concentrations, and expression of steroidogenesis-related genes in the ovary were examined. Exposure to 30 µg fadrozole/L significantly reduced plasma E2 and vitellogenin concentrations after just 1 d and those effects persisted throughout 28 d of exposure. In contrast, ex vivo E2 production was similar to that of controls on d 8-28 of exposure, while transcripts coding for aromatase and follicle-stimulating hormone receptor were elevated, suggesting a putative compensatory response. Following cessation of fadrozole delivery ex vivo E2 and plasma E2 concentrations exceeded and then recovered to control levels, but plasma vitellogenin concentration did not return to control levels, even after 20 d of depuration. Collectively these data provide several new insights as to the nature and time-course of adaptive responses of the HPG axis of a model vertebrate and its response to aromatase inhibitors, a class of endocrine-active chemicals of regulatory concern. *The contents of this abstract neither constitute nor reflect official US EPA policy.*

**WP208 Molecular Characterization of Hemoglobin Protein in Larvae of 4th Instar Chironomidae: A Promising Biomarker for Evaluating Environmental Quality** J. Oh, Seton Hall University / Department of Biological Sciences; C.S. Bentivegna, Seton Hall University / Department of Biological Sciences, Seton Hall University / Biology department. Larvae



of 4th instar Chironomidae or chironomids are a convenient animal model for studying environmental quality. In order to generate a useful biomarker, we have been attempting to characterize various molecular responses exhibited by chironomid upon a change in their environment – especially upon exposure to heavy metals, which are frequent toxic pollutants found in urban environments. Previous results indicated that hemoglobin (Hb) proteins of chironomid – major proteins found in their hemolymph – are an appropriate biomarker for evaluating environmental quality because they are modulated by heavy metals like cadmium (Cd). Our data suggested that low molecular weight proteins (4-6 KDa), observed on SDS PAGE in the control group, were potentially representing normal degradation processes by indigenous proteases. However, upon exposure to low concentrations of Cd, varying Hb protein profiles were observed – either disappearance or decreased intensity of the low molecular weight proteins – suggesting that the normal degradation process might have been inhibited by the presence of Cd. In addition, it was evidenced by LCMS that the low molecular weight proteins were identified as possible fragmented products of Hb genes IV and VII. In this study, we used trypsin (serine) and cathepsin D (aspartic) to further investigate the degradation process of Hb proteins in larvae of *Chironomus riparius*.

**WP209 Monitoring of core biomarkers in field benthic fish to assess the environmental impact in the Southern Gulf of Mexico** J. Rubio-Pina, A. Fuentes, V. Patino-Suarez, M. Del Rio, CINVESTAV / Aquatic Toxicology; O. Zapata-Perez, Cinvestav Unidad Merida / Secretaria Academica. The assessment of marine ecosystems is an important aspect of environmental protection. For this reason, a way to establish the connection between the levels of pollutants and their harmful effects on organisms is the monitoring programs that incorporate biological tools such as biomarkers. A two-year biomonitoring was conducted between 2009-2011 in the southern Gulf of Mexico, using benthic fish *Ariopsis felis* (hardhead) and *Scyrium gunteri* (Flatfish) as a sentinel species. Three hundred and forty fish (125 *Ariopsis felis* and 215 *Scyrium gunteri*) were collected in 38 sampling sites along the Gulf of Mexico including areas of oil exploitation. Quantitative differences on liver mRNA levels of central biomarkers such as cytochrome P450 1A (CYP1A), glutathione S-transferase (GST), metallothionein (MT) and vitellogenin (VTG) were used as markers of exposure to polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and toxic metals. At the molecular level showed that the highest expression of the gene CYP1A, GST, MT and VTG were detected in fish collected near the coast of Tabasco (Dos Bocas), and the exclusion zone in the oil region. The association between gene expression and Aromatic Hydrocarbons in the liver of fish was low, although its main association was with 2,6-dimethyl-naphthalene and 2,3-dimethyl-naphthalene, indicating that organisms have a higher metabolic activity related to the processing and disposal of low molecular weight PAHs of petrogenic origin. Biomarkers high expressions in the exclusion zone, suggests that the organisms are responding to the presence of other types of chemical compounds or mixtures, such as dispersants, detergents or emulsifiers with the characteristic to cause oxidative effects and endocrine cells in the agencies. Finally, in this study the levels of the biomarkers evaluated in the livers of fish in 2011 were higher than those detected in previous oceanographic campaign 2009. The results are important for determining biological responses or effects may be related to exposure to one or several chemical compounds from the environment.

**WP210 Vitellogenin detection in rainbow trout (*Oncorhynchus mykiss*) using cryotechniques and immunofluorescence** J. Guchardi, University of Ontario Institute of Technology / Faculty of Science, Aquatic Toxicology, University of Ontario Institute of Technology / Senior Research Laboratory Technician; R. Krause, University of Ontario Institute of Technology / Faculty of Science, Aquatic Toxicology; R. Orrego, University of Ontario Institute of Technology / Faculty of Science, University of Ontario Institute of Technology / Faculty of Science, Aquatic Toxicology, University of Ontario Institute of Technology / post doctoral fellow; D. Holdway, University of Ontario Institute of Technology / Faculty of Science, Aquatic Toxicology, University of Ontario Inst. of Tech / Faculty of Science. Vitellogenin (VTG) is an egg yolk precursor protein which can be used as a biomarker of exposure to estrogenic xenobiotics found in the environment. Produced in the liver, this protein can be detected in the blood using an enzyme linked immunosorbent assay (ELISA). Once in the gonadal tissue, it can be detected within the cells using immunofluorescence (a specific type of immunohistochemistry, IHC) in paraffin embedded or frozen tissue sections.

Detection using traditional paraffin histology has been criticized due to extensive tissue processing which can compromise antigen integrity. Using immunofluorescence in conjunction with “frozen section histology” can preserve antigens for antibody recognition. This project analyzed gonadal tissue of twelve sexually mature rainbow trout using paraffin and frozen section histology and immunofluorescence. The two techniques of tissue and section preparation were compared for efficacy and section quality. The method for detecting VTG with immunofluorescence on frozen sections was optimized. Results were qualitatively compared to blood VTG levels, as well as the known sex and maturation stage of each fish. Frozen section histology proved to be a useful technique yielding sections which were comparable to paraffin section histology. Immunofluorescence was effective at detecting VTG within oocytes with the fluorescence intensity correlating to oocyte maturity. Frozen section histology and immunofluorescence will be useful for detecting VTG induction in fresh gonadal tissue of rainbow trout exposed to estrogenic xenobiotics in both environmental and laboratory settings.

**WP211 Bile and Liver Metallothionein Comparisons in Fish Sublethally Exposed to Copper: Is Bile Analysis More Adequate Regarding Metal Exposure?** R.A. Hauser-Davis, UNICAMP / Chemistry; B. Tuton, Puc-Rio; R. Zioli, UNIRIO; T. Saint-Pierre, R. de Campos, Puc-Rio; M. Zezzi Arruda, UNICAMP. Metallothioneins (MTs) are validated biomarkers for environmental metal exposure and are routinely analyzed in liver and other organs in fish. However, it has recently been discovered that MTs are also excreted in fish bile, and that they also follow the same trend as hepatic MT in environmental contamination situations. Thus, it is of interest to further study these proteins in bile, in order to characterize their metal binding behaviour, as well as their behaviour in different metal exposure situations. MTs in Nile Tilapia (*Oreochromis niloticus*) bile obtained from a reference site and exposed to sub-lethal copper concentrations were quantified by spectrophotometry, with a modified Ellman's reaction and the MT metal binding profile was characterized by size exclusion high performance liquid chromatography coupled to ICP-MS (SEC-HPLC-ICP-MS). Results show that liver MT is approximately 250-fold higher than bile MT from the reference group. Differences between MT profiles in the control and exposed group were observed for both liver and bile, indicating differential metal binding behaviour by liver MT in comparison to bile MT. Biliary MT excretion showed statistically significant differences among the control and exposed group, while the same did not happen with liver MT. Thus, we propose that the analysis of bile MTs are more adequate than liver MTs if the aim is to observe recent metal exposure, since the rapid excretion of biliary MT indicates that organism responses are more quickly observed in bile, while liver takes more time to accumulate MT before excretion from the body.

**WP212 The pregnane X receptor (PXR) and the regulation of P-glycoprotein functional activity in zebrafish** J. Jackson, Simon Fraser University / Department of Biological Sciences; C. Kennedy, Simon Fraser University. Fish and other aquatic organisms often inhabit polluted environments and rely on cellular mechanisms of defense to reduce the potential for adverse effects associated with exposure to environmental toxicants. One such mechanism involves the ATP-binding cassette (ABC) transporter, P-glycoprotein (P-gp), which actively pumps xenobiotics and their metabolites from the cells of vulnerable fish tissues such as the liver, gills, and brain. In mice and rats, gene expression of P-gp and the cytochrome P450 monooxygenase, CYP3A, is controlled by the pregnane X receptor (PXR) – a nuclear receptor directly activated by a diverse range of xenobiotics. However, the mechanisms underlying P-gp expression and functional activity have not yet been described in fish. To examine the role of PXR in regulating fish P-gp functional activity, *Danio rerio* (zebrafish) were intraperitoneally injected with 20mg/kg of the model PXR activator and synthetic steroid, pregnenolone 16 $\alpha$ -carbonitril (PCN), and a combined equimolar dose of PCN with an established PXR inhibitor, ketoconazole (KTC) (20mg/kg PCN + 31mg/kg KTC). P-gp functional activity will be assessed using an *in vivo* rhodamine 123 (R123) efflux assay. R123 is a fluorescent dye and P-gp substrate, thus fluorometric detection of liver R123 will indicate P-gp activity in fish. Results are presented as relative R123 fluorescence between control, PCN, and PCN + KTC treated fish at 0, 1, 2, 4, 8, and 24 hours post R123 exposure. Real time quantitative PCR (RT-PCR) was used to examine the relative gene expression of P-gp, CYP3A, and PXR across treatment groups. This study will be the first to link the PXR-mediated regulation of fish



P-gp at the molecular level to P-gp functional activity at the cellular level, thus providing clues to how fish respond to a variety of chemicals in their environment.

**WP213 Transfected vs. native: The potential for conflicting measurements of endocrine activity from different cell lines** S.M. Brander, Bodega Marine Lab, University of California, Davis / Environmental Toxicology, / Department of Biology & Marine Biology; G. He, University of California, Davis / Dept. Environmental Toxicology; R. Hudson-Davies, University of California, Davis / School of Veterinary Medicine, Dept. Anatomy, Physiology and Cell Biology; R.E. Connon, University of California – Davis / School of Veterinary Medicine, Aquatic Toxicology Laboratory, University of California, Davis / Anatomy, Physiology and Cell biology; M.S. Denison, University of California, Davis / Dept. Environmental Toxicology. The U.S. Environment Protection Agency has recently recommended that endocrine disruptor screening be conducted on contaminants of emerging concern (CECs), and that wastewater be monitored to assess compounds with potential endocrine activity entering aquatic ecosystems. CECs include the synthetic pyrethroid bifenthrin; due to its broad usage and aquatic toxicity range, and the pharmaceutical ibuprofen, which is not fully metabolized by humans and can enter the sewage system as the parent compound or metabolites. Bifenthrin has been shown to have both estrogenic and anti-estrogenic properties *in vitro* and *in vivo*, and ibuprofen is known to mediate levels of prostaglandin, a lipid mediator that plays multiple roles in reproduction. High throughput screening utilizing cell lines that evaluate the potential for estrogenic or androgenic activity is now a preferred method of prioritizing CECs for further assessment; however, results from cell lines of different origins are often conflicting. Conflicting results may occur between assays such as the CALUX that natively express the estrogen or androgen receptor and those such as the yeast estrogen / androgen screen (YES / YAS) that are transfected with these receptors. Additionally different responses may be seen depending on the concentration used, considering the sensitivity of endocrine responses. As such, to date the majority of *in vitro* assays have used concentrations of bifenthrin and ibuprofen that are much higher than levels detected in the environment. We will present comparative results from tests conducted on environmentally relevant concentrations of bifenthrin and ibuprofen in two *in vitro* systems, the CALUX and YES/ YAS, determining their contribution to estrogenic and androgenic activity in aquatic ecosystems, as well as differences in the quantitation of estrogenic or androgenic potency between these two assay types.

**WP214 Acute and Chronic Aquatic Toxicity and Bioconcentration Assessment of a Perfluorinated Aliphatic Carboxylic Acid, (C<sub>6</sub>HF<sub>11</sub>O<sub>3</sub>.H<sub>3</sub>N)** R. Hoke, DuPont, Haskell Global Centers, E.I. DuPont de Nemours and Co., E.I. DuPont de Nemours and Co. / Haskell Global Centers for Health and Environment; B. Ferrell, T. Sloman, P. Bloxham, W. Buxton, E. I. DuPont de Nemours and Co.. Driven by a commitment to curtail the use of long-chain perfluoroalkyl acids (PFAAs), the fluoropolymer manufacturing industry is moving to technologies with more favorable toxicological and environmental profiles. As part of the environmental product stewardship assessment and premanufacture notification (PMN) process, we conducted acute and chronic aquatic toxicity tests to evaluate the toxicity of propanoic acid, 2,3,3,3-tetrafluoro-2-(1,1,2,2,3,3,3,3-heptafluoropropoxy)-, ammonium salt (C<sub>6</sub>HF<sub>11</sub>O<sub>3</sub>.H<sub>3</sub>N) to the cladoceran, *Daphnia magna*, the green alga, *Pseudokirchneriella subcapitata*, and the rainbow trout, *Oncorhynchus mykiss*. In addition, testing with the common carp, *Cyprinus carpio*, was conducted to determine the bioconcentration potential of the acid form of the compound. The results of the aquatic toxicity studies indicated the substance is of low concern from an acute and chronic aquatic hazard classification standpoint. Similarly, data from the bioconcentration study with carp demonstrate that the substance is not a bioconcentration concern for aquatic organisms.

**WP215 Acute and Chronic Aquatic Toxicity and Bioconcentration Screening of 5:3 Polyfluorinated Acid** R. Hoke, DuPont, Haskell Global Centers, E.I. DuPont de Nemours and Co., E.I. DuPont de Nemours and Co. / Haskell Global Centers for Health and Environment; B. Ferrell, T. Sloman, P. Bloxham, R. Buck, E. I. DuPont de Nemours and Co. Polyfluorinated carboxylic acids have been identified in various environmental matrices from their direct emissions or as degradation products of fluorotelomer alcohols. The X:3 polyfluorinated acids [F(CF<sub>2</sub>)<sub>x</sub>CH<sub>2</sub>CH<sub>2</sub>CO<sub>2</sub>H, x=5,7] are degradation products from fluorotelomer alcohols [FTOHs,

F(CF<sub>2</sub>)<sub>n</sub>CH<sub>2</sub>CH<sub>2</sub>OH]. There is a need to better understand the environmental fate and effects of these degradation products. We conducted acute and chronic aquatic toxicity tests to evaluate the toxicity of the 5:3 polyfluorinated acid, C<sub>5</sub>F<sub>11</sub>CH<sub>2</sub>CH<sub>2</sub>COOH, to the cladoceran, *Daphnia magna*, the green alga, *Pseudokirchneriella subcapitata*, and the rainbow trout, *Oncorhynchus mykiss*, as part of an on-going product stewardship program. In addition, tissue residue measurements in rainbow trout were conducted as part of the early-life stage study as a screen for bioconcentration potential. The 5:3 polyfluorinated acid is of low to medium concern from an acute and chronic hazard classification standpoint. When compared to potential aquatic environmental exposure concentrations, the hazard data suggests that the 5:3 acid poses little risk for the aquatic environment. Similarly, the bioconcentration screening data suggest that the 5:3 acid is not a bioconcentration concern for aquatic organisms.

**WP216 Acute Toxicity of Hydrazine to Freshwater Fish and Invertebrates** D.G. Poirier, Ontario Ministry of the Environment / Laboratory Services Branch; R. Chong-Kit, Ontario Ministry of the Environment; J. Sowa, University of Guelph. Hydrazine (N<sub>2</sub>H<sub>4</sub>) is a highly reactive, basic material. It is used in Ontario as an oxygen scavenger in boiler feed waters, for the removal of halogens in wastewaters, as an intermediate in polymerization reactions and as a component in rocket fuel. Hydrazine is frequently accidentally discharged to surface waters in Ontario, and little is known of toxic effects on indigenous species. In published studies, the toxicity of hydrazine varies at least 100 fold among aquatic species. In Ontario, there have been no fewer than five hydrazine discharges from industrial facilities in the past five years. To support spills investigations and surface water standards development within the province the Ontario Ministry of the Environment (OMOE) Aquatic Toxicology Unit (ATU) investigated the acute lethal toxicity of hydrazine to four species of cold water fish (Rainbow trout, Round Whitefish, Lake trout and Arctic Char), one warm water fish (Fathead Minnow), and five species of aquatic invertebrates (*Daphnia magna*, *Chironomus dilutus*, *Hexagenia* spp., *Lumbriculus variegatus* and *Hyalella azteca*). While invertebrate species were tested at their respective optimal temperatures, toxicity of hydrazine to all fish species was assessed at 5, 10 and 15°C, after culture pre-acclimation to temperature. Where available, testing followed standardized test methodologies, and species for which no standardized methodologies are available were tested using best scientific practices. Due to the structural nature of this compound it is thought to be highly reactive and the stability of this compound in test vessels over time was also investigated.

**WP217 Characteristics and Sorption Kinetics of Explosives in Sediments** T. Ariyaratna, University of Connecticut / Dept. of Marine Sciences; P. Vlahos, C. Tobias, University of Connecticut / Department of Marine Sciences. The explosive compounds, 2,4,6-trinitrotoluene (TNT) and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) are synthesized globally, mainly as military munitions, and have become priority pollutants of concern. As coastal zones and estuaries are commonly impacted zones it is important to understand the fate and transport of these compounds in these environments. Here we present studies focused on the sorption kinetics of TNT and RDX onto upper-intertidal sand (as a low carbon containing sediment), intertidal silt (as a finer, high carbon containing sediment) and freshwater silt. Sediments were classified based on particle size distribution curves, x-ray diffraction analysis, organic carbon, nitrogen and elemental sulfur compositions. Organic carbon and nitrogen were higher in finer silty sediments and elemental sulfur was elevated in marine silt and sand but very low in freshwater silt. Abiotic sediment sorption tests were carried out at 23°C, 15°C and 4°C by spiking TNT and RDX solutions into sediment slurries. Experiments were conducted over two weeks and time series data showed clear relationships between sediment sorption of explosives and temperature. Sorption rates ranged from 2.56 to 8.76 and 0.62 to 11.10 nmol g<sup>-1</sup> hr<sup>-1</sup> for TNT and RDX respectively and were inversely proportional to temperature for all sediment types. Sediments equilibrated with water in just over an hour. Sorption rates of TNT and RDX were not significantly different across sediment types. Steady-state partition constants ranged between 1.0 and 4.6 for RDX and 1.0 to 2.8 for TNT on sand and silt respectively. Biotic experiments in sediments were conducted to quantify rates of degradation and to isolate pathways using both non-labeled and N<sup>15</sup> labeled parent compounds. These experiments will be extended in order to include possible effects on rates of other physico-chemical parameters such

as pH, salinity, dissolved oxygen, dissolved organic matter, iron and sulfide on sediment sorption and degradation of TNT and RDX.

**WP218 Developmental and Reproductive Effects of Triclocarban on Japanese Medaka (*Oryzias latipes*)** L. Flores, University of California- Davis / Veterinary Medicine: Anatomy, Physiology and Cell Biology; T. Kurobe, S. Teh, University of California- Davis. Triclocarban (TCC; 3,4,4'-trichlorocarbanilide) is a common antibacterial additive in personal care products. As a result of extensive human usage and incomplete removal by wastewater treatment plants, TCC has been detected at levels as high as 6.75 µg/L in surface waters in the United States. TCC has been overlooked as a contaminant but due to its frequent detection in US surface waters, it has become a matter of widespread concern. The objective of this study is to investigate the chronic effects of TCC using the medaka fish model (*Oryzias latipes*). A full life cycle chronic toxicity test was initiated by exposing 1-day-old embryos to 5 µg/L of TCC continuously for 5 months until full maturation. Various endpoints were measured. Growth was assessed by weight and length at multiple time points. Differences in weight and lengths between exposed and control fish were observed. Expression of vitellogenin, a female egg yolk precursor protein, was measured to assess the reproductive health of males. Significant up-regulation of vitellogenin was observed in male fish exposed to TCC at 5 months, indicating potential estrogenic effects due to TCC exposure. Gene expression analysis by quantitative PCR also supports activation of estrogen-related genes, which are responsible for female reproduction. In addition, fertility and fecundity of chronically exposed fish were evaluated through crossbreeding experiments. Fertility in pairs with one or both exposed parents was significantly lower than pairs with both control parents suggesting impaired reproduction from an environmentally relevant level of TCC exposure. This is the first study evaluating the life-cycle effects of Triclocarban on medaka. These findings observed at the molecular and phenotypic levels significantly advance the understanding of adverse effects due to TCC exposure. Results from this study will be useful in predicting population effects of TCC and provide insight on the environmental impact of TCC.

**WP219 Developmental toxicity of oxybenzone in zebrafish** G. Rodriguez-Fuentes, F. Rubio-Escalante, E. Norena-Barroso, Universidad Nacional Autonoma de Mexico / Unidad de Química Sisal. Sunscreens and their bioactive metabolites are continuously being introduced into the aquatic environment as complex mixtures, indirectly by either untreated or treated sewage or directly from recreational activities as bathing and swimming. As most of the UV filters are lipophilic, they can accumulate in the environment and aquatic animals, causing adverse effects. Oxybenzone (benzophenone-3) is an organic compound used in sunscreen; it absorbs UVB and short wave UVA rays. Studies on the effects of oxybenzone have almost entirely focused on its estrogenicity, but there are some reports that indicated that this compound may have other important toxic effects. In this study we evaluated the toxicity of oxybenzone during early stages of zebrafish development. Zebrafish embryos were exposed to increasing concentrations (0.1 to 1000 ng/ml) of oxybenzone. Neurotoxicity, oxidative stress and estrogenicity were evaluated in zebrafish 72 hours post fertilization. Endpoints used for assessing developmental toxicity included embryo survival, hatching rate, heartbeat pericardial sac edema and yolk sac edema.

**WP220 Effect of HR96 inhibitors such as Triclosan and Polyunsaturated Fatty Acids on Toxicant Sensitivity in *Daphnia*** N. Sengupta, Clemson University / Environmental Toxicology Program, Department of Biological Sciences, Clemson University; W.S. Baldwin, Institute of Environmental Toxicology / Department of Biological Sciences. *Daphnia* are small planktonic crustaceans, widely used for toxicology testing, and the first crustacean to have its genome fully sequenced. HR96 is a promiscuous xenobiotic and endobiotic nuclear receptor in *Daphnia* and an ortholog of CAR and PXR that are found in vertebrates. HR96 is activated by most toxicants such as atrazine, chlorpyrifos, pyriproxyfen, and estradiol, as well as n-6 and n-9 (omega-6 and 9) fatty acids. However, HR96 is also inhibited by several chemicals including Triclosan and some n-3 (omega-3) unsaturated fatty acids. We hypothesize that inhibitors of HR96 could potentially block a protective response to toxicants and in turn cause synergistic toxicity when coupled with other chemicals. We have performed acute toxicity tests with triclosan, atrazine, docosahexaenoic acid (DHA) (n-3), linoleic acid (n-6), and oleic acid (n-9). LC50s to the polyunsaturated fatty acids are typically about 10 mM, with DHA showing the greatest toxicity. Triclosan and

atrazine have LC50's of 0.84 and 78 mM, respectively. Chronic treatment of adults at 2 and 4 mM DHA decreased survival 40%, and 0.7 mM triclosan decreased survival 100%. Chronic toxicity tests also demonstrated that neither Triclosan nor the fatty acids DHA and linoleic acid significantly perturbed reproduction. Therefore these chemicals could make excellent choices for determining whether they elicit synergistic toxicity when exposed to mixtures. We have not examined atrazine yet. Because we hypothesize that HR96 inhibitors such as Triclosan could block the physiological responses of daphnids to respond to toxicant insult, we will examine the effects of Triclosan and other HR96 inhibitors on the sensitivity of other chemicals such as atrazine. Activation or inhibition of this promiscuous receptor could eventually impact survival and reproduction rates in *Daphnia magna*. Overall, our data may ultimately indicate a potential mechanism by which diet or specific xenobiotics increase an individual's sensitivity to specific chemicals or mixtures of chemicals.

**WP221 Effects of chronic exposure to perfluorodecanoic acid and perfluorotridecanoic acid in *Daphnia magna* and Zebrafish and endocrine disruption potential** A. Jo, Seoul National University / Environmental Health, School of Public Health, Seoul National University / Department of Environmental Health; K. Ji, University of Saskatchewan / Biomedical Veterinary Sciences and Toxicology Centre, University of Saskatchewan; B. Ahn, Chungbuk National University / College of Veterinary Medicine; K. Choi, Seoul National University / School of Public Health. Perfluorinated compounds (PFCs) have been frequently detected in both the environment and biota, and their potential toxicities are of concern. But most of the studies have been focused on PFOS and PFOA. Endocrine disruption potentials and the chronic toxicity of the compounds have not been fully understood. The aim of present study was to investigate effects of long time exposure to PFDA or PFTTrDA employing *Daphnia magna* and zebrafish and to understand potential endocrine disruption mechanisms. *D. magna* were cultured in M4 following OECD TG 202 and survival, reproduction, and growth assessed. Each neonate of 10 replicates was exposed to PFDA (0, 1.95, 7.81, 31.3, 125, and 500 µg/L) and PFTTrDA (0, 0.78, 3.13, 12.5, 50, and 200 µg/L). The test solution was renewed 3 times per week and newborn neonates were counted and removed daily. Four replicates with 10 fish eggs each were exposed to various concentrations of PFDA or PFTTrDA (0, 0.01, 0.1, 1, and 10 mg/L). Survival was recorded at 17, 34, 61 and 120 day post fertilization (dpf). At the end of exposure, concentrations of sex hormones (estradiol, testosterone and 11-keto testosterone) were measured by competitive enzyme-linked immunosorbent assay (ELISA) kit. Transcription of aromatase, CYP17, ERα and ERβ gene was quantified using quantitative real-time PCR assay. *D. magna* exposed to PFTTrDA showed reduced survival at concentrations as low as 50 µg/L. First day of reproduction was significantly decreased at 100 µg/L PFTTrDA. Growth rate was significantly declined at 1.95, 7.81, 31.3, 125 and 500 µg/L in PFDA. The concentrations of 11-KT and T in plasma were significantly decreased at 0.1 mg/L PFTTrDA in male fish. The T/E2 and 11-KT/E2 ratio were significantly decreased at 0.1 mg/L PFTTrDA. Aromatase was significantly declined at 0.01 mg/L and 0.1 mg/L PFTTrDA in male. PFDA exposure, however, did not show statistically significant difference. Long term exposure to PFTTrDA (and PFDA to lesser extent) affects survival of fish, and alters synthesis of sex steroid hormone and related gene transcription in fish.

**WP222 Evaluation of Selected Substances in Consumer Products for PBT Characteristics** L.R. Gneiding, AMEC Environment & Infrastructure, Inc.. Consumer products are used for the most part under the pretense that they are safe for human health as well as for the environment. An evaluation of the persistence, bioaccumulative, and toxicological (PBT) properties associated with selected substances found in commercially available product will be completed to provide data for characterizing consumer product safety.

**WP223 Mixture toxicity of triclosan and triclocarban in *Daphnia magna*** K. Albanese, The Ohio State University / Environmental Science Graduate Program; R. Lanno, Ohio State University / Department of Evolution, Ecology, and Organismal Biology; Y. Chin, The Ohio State University / Department of Geological Sciences, The Ohio State University / School of Earth Sciences; C. Hadad, The Ohio State University / Department of Chemistry. Triclosan (2,4,4'-trichloro-2'-hydroxydiphenyl ether) and triclocarban (3,4,4'-trichlorocarbanilide) are two antimicrobial compounds that are commonly found in many household products, including hand soaps, toothpaste, and cleaning supplies. Usage of these compounds in personal



care products has escalated over the last few decades, leading to a dramatic increase in their environmental presence. This presents an array of potential problems because both compounds have the potential to be transformed in the environment into chemicals that may be potential carcinogens. Studies have shown that triclosan converts to various dioxins through reactions with light in surface waters. In wastewater treatment facilities where chlorination is used, more highly chlorinated forms of dioxin have been seen. In dioxins, a higher degree of chlorination usually means a more carcinogenic compound. In addition to these concerns, triclosan itself has been noted to be an endocrine disruptor in various organisms. Much less is known about the environmental fate of triclocarban, but some studies have linked it to carcinogenicity through the creation of ortho quinone imines from triclocarban and its degradation products. Ortho quinone imines are believed to cause carcinogenic effects due to their interactions with proteins, DNA, and other cellular components. While little has been studied about the environmental fate of triclocarban, even less has been studied about the effects of triclosan and triclocarban in mixtures on organisms, even though they are most often detected concurrently in natural water samples. The goal of this project is to determine if any interactions between these two compounds increase toxicity. Individual and mixture toxicity tests will be performed on *Daphnia magna* at varying concentrations of each compound. Photochemistry and computational chemistry will be used to determine reaction mechanisms, intermediates, and phototransformation products of these compounds independently and concurrently in water. All samples will be analyzed by HPLC-MS/MS.

**WP224 Removal of bisphenol A related compounds by *Portulaca oleracea* root and horse radish peroxidase** K. Arizono, Prefectural University of Kumamoto / Faculty of Env. & Symbiotic Science; N. Takemoto, Graduate School of Engineering, Nagasaki Institute of Applied Science; H. Miyasaka, The Kansai Electric Power Co., / Environmental Research Center; H. Ji, Prefectural University of Kumamoto; Y. Ishibashi, Prefectural University of Kumamoto / Faculty of Environmental and Symbiotic Science. Bisphenol A (2,2-bis(4-hydroxyphenyl)propane, BPA) is widely used as an ingredient in the manufacture of epoxy carbonate, poly carbonate and polyester styrene. Recently, some chemicals which are structurally similar to BPA (BPA related compounds: BPs) are coming to be used and it is reported that they have estrogenic activity on aquatic organisms. In this study, we determined the ability of *Portulaca oleracea* root and horse radish peroxidase (HRP) to remove 5 BPs (BPA, BPAF, BPZ, TDP and MBP) from water samples. When the initial BPs concentrations were 1 ppm, as same as BPA, BPZ, TDP and MBP were indicated removable ability at over 80% within 48hr, but BPAF could not be removed. The case of HRP, BPZ, TDP and MBP were indicated removable ability at over 45% within 60 min, but BPAF was removed 11.7 %.

**WP225 Species sensitivity to fluoranthene photo-induced toxicity and toxicokinetics in sheepshead minnow (*Cyprinodon variegatus*) and zebrafish (*Danio rerio*)** A.M. Willis, Miami University / Zoology, Miami University; A.J. Tucker, L. Smith, Miami University / Zoology; J.T. Oris, Miami University / Department of Zoology, Miami University / Office for the Advancement of Research & Scholarship. Aquatic organisms are exposed to polycyclic aromatic hydrocarbons (PAHs) through various anthropogenic sources, including atmospheric deposition, runoff, and oil spills. A number of PAHs can absorb energy from solar ultraviolet radiation (UVR), causing photo-induced toxicity by generating reactive oxygen species (ROS) that can bind to cellular components leading to adverse effects in organisms including tissue, cell, DNA, and biomolecular oxidative damage. Photo-induced toxicity leads to an increase in PAH toxicity up to 10,000-fold in aquatic organisms compared to toxicity in the absence of UVR, particularly during susceptible life stages such as early translucent life stages. Levels of toxicity are determined primarily by the body concentration of phototoxic PAH and intensity of UVR. Species-specific sensitivities to photo-induced toxicity can be related to differences in bioaccumulation of PAH or to physiological adaptations with respect to ROS stressors. In this study, the toxicokinetics of a phototoxic PAH, fluoranthene, were investigated in the sheepshead minnow (*Cyprinodon variegatus*) and zebrafish (*Danio rerio*) during larval stages. Uptake (*ku*) and elimination (*ke*) rate constants were estimated for each organism exposed to fluoranthene in the absence of UVR. Organisms were also exposed to fluoranthene in the presence of UVR for 96 hours to derive estimates of the medial lethal concentrations (LC<sub>50s</sub>) for each species. Zebrafish were more sensitive than sheepshead minnows to fluoranthene

and UVR – 20 µg fluoranthene/L caused 100% mortality in zebrafish and < 1.0% mortality in sheepshead. Whereas there were no differences in *ke* values between species, zebrafish had a significantly greater *ku* value compared to sheepshead. Thus the bioconcentration factor for zebrafish (5,353) was significantly greater compared to sheepshead (1,081). While there are variations in physiological adaptations that may cause zebrafish to be more susceptible to ROS damage, such as differences in pigmentation, differences in accumulation rates of fluoranthene appear to explain the majority of the species-specific toxicity values. Assessments of photo-toxic compounds that are based on either measured or predicted body residue concentrations will be able to account for a significant proportion of species-specific differences in toxicity levels and reduce uncertainty in risk assessments of these compounds.

**WP226 Toxicity and Bioaccumulation Potential of a Perfluorinated Aliphatic Carboxylic Acid in an Avian Reproductive Study with the Northern Bobwhite Quail** R. Hoke, DuPont, Haskell Global Centers, E.I. DuPont de Nemours and Co., E.I. DuPont de Nemours and Co. / Haskell Global Centers for Health and Environment; D. Temple, K. Martin, Wildlife International, LTD; W. Buxton, E. I. DuPont de Nemours and Co.. Driven by a commitment to curtail the use of long-chain perfluoroalkyl acids (PFAAs), the fluoropolymer manufacturing industry is moving to technologies with more favorable toxicological and environmental profiles. As part of the environmental product stewardship assessment and pre-manufacture notification (PMN) process, we conducted a chronic avian reproduction study with the northern bobwhite quail, *Colinus virginianus* to evaluate the chronic toxicity of propanoic acid, 2,3,3,3-tetrafluoro-2-(1,1,2,2,3,3,3,3-heptafluoropropoxy)-, ammonium salt (C<sub>6</sub>HF<sub>11</sub>O<sub>3</sub>·H<sub>3</sub>N). In addition, various tissues were sampled to evaluate potential dietary bioaccumulation of the substance. The results of the avian reproduction study demonstrated no treatment-related effects at 1000 ppm (84.5 mg/kg/day), the highest dose tested in the study. Evaluation of the various tissues collected during the study also demonstrated that the substance is not of concern for potential dietary bioaccumulation by terrestrial wildlife.

**WP227 Toxicity of Ionic Liquids to Wetland and Agricultural Plants** B. Brown, H. Craytor, S. Gilmore, J. Pierce, R. Afzal-Asr, V. Cassel, C. Howard, Kennesaw State University; H. Sutton, Kennesaw State University / Department of Bio. & Phys. Sciences. Ionic liquids (ILs) are liquid salts being explored for a variety of industrial applications. Their properties of low volatility, lack of flammability, and ease of being manipulated (by altering the cation and anion components) make them a potentially good alternative to current solvents. As of yet, ILs have apparently not been released into aquatic systems, but as use increases, so does the potential for such releases to occur. The effects of the ionic liquids 1-ethyl-3-methylimidazolium chloride, 1-butyl-3-methylimidazolium chloride, 1-hexyl-3-methylimidazolium chloride and 1-octyl-3-methylimidazolium chloride were assessed with respect to the growth rates of a variety of aquatic and wetland plants, as well as two representative agricultural species. Frond growth was measured as the endpoint for the floating aquatic plant *Lemna gibba* (duckweed). For the agricultural species *Raphanus sativus* (radish) and *Lactuca sativa* (lettuce), as well as the native wetland plants *Asclepias incarnata* (swamp milkweed), *Actinomeris alternifolia* (wingstem), and others, endpoints measured were shoot and root length and shoot and root mass. Alkyl chain length was found to affect toxicity, with the lowest toxicity to *L. gibba* occurring with the shortest alkyl chain (1-ethyl-3-methylimidazolium chloride; IC<sub>50</sub> = 45.5mg/L), while toxicity increased with increasing chain length to an IC<sub>50</sub> of 0.117mg/L for the 1-octyl-3-methylimidazolium chloride. Initial results suggest variation in sensitivity of endpoints between the agricultural species and the native wetland species, for example in *A. alternifolia* exposed to 1-hexyl-3-methylimidazolium chloride, shoot length was the most sensitive endpoint, whereas root mass was the most sensitive endpoint for *R. sativus*.

**WP228 Toxicological effects of benzo[a]pyrene (BaP) in p53 and BCRA2 gene expression levels in *Oreochromis niloticus*** V. Patino-Suarez, J. Rubio-Pina, CINVESTAV / Aquatic Toxicology; O. Zapata-Perez, Cinvestav Unidad Merida / Secretaria Académica. Polycyclic aromatic hydrocarbons (PAHs) are common toxic pollutants found in the aquatic environment. It is well known that benzo[a]pyrene (BaP) is a carcinogenic compound, so the assessment of their impact on life is of considerable concern. In this research was studied the effect of benzo[a]pyrene (BaP) on p53 and BCRA2 mRNA level in male juveniles of *Oreochromis niloticus*. To



evaluated the gene expression, thirty tilapias were injected *ip* with a single dose of corn oil (vehicle) with benzo[a]pyrene (BaP) (20 mg kg<sup>-1</sup> of body weight) and were sacrificed at 0, 3, 6, 9, 12, 24, 48, 72, 96, 120 and 124 h. Fish livers were removed and frizzed at -120°C. A 563, 320 and 450 pb fragments of tilapia (CYP1A, P53, and BCRA2 cDNA, respectively) were cloned, sequenced, and compared with CYP1A, P53, and BCRA2 reported sequences in the GeneBank DNA database. Total RNA was extracted from livers and CYP1A, p53 and BCRA2 gene expressions were assessed using specific Taqman primers and by Real Time-PCR. Gene expression results showed significant differences between CYP1A, p53 and BCRA2 levels according to different exposure times. Further analyses on the genes modulated by these pathways are needed to understand adaptation responses in tropical fishes.

**WP229 Toxicoproteomic analysis of *Daphnia magna* to Pharmaceuticals, Caffeine and Ibuprofen** N. Hong, Chonbuk National University / Bioprocess Engineering; T. Le, Chonbuk National University / Dept. of Bioprocessing Engineering; Y. Kim, Chungbuk National University; J. Min, Chonbuk National University. Pharmaceuticals have been produced and consumed increasingly in the world, but they may have some adverse impacts on our environments. In this study, the impacts of caffeine and ibuprofen were examined through toxicity assay based on toxicoproteomic analysis. The acute (48h) and chronic toxicity (21d) assay using *Daphnia magna* as animal model were performed. The lethal concentration with 50% mortality (LC50) of caffeine and ibuprofen was determined as about 445.3 mg/l and 91.5 mg/l respectively. In the chronic toxicity test, several small concentrations of pharmaceuticals such as 1%, 2%, 10%, 20% LC50 were used to expose to organisms for 21 days. In order to examine the effect of these pharmaceutical on molecular responses in organism, the gene expression level of 5 different biomarkers in *Daphnia magna* (e.g., *Dhb*, *Vtg*, *Arnt*, *CYP4* and *CYP314*) were investigated after 48h and 21d exposure to the testing pharmaceuticals. Additionally the acute and chronic test showed that 5 genes have different responsive patterns when *Daphnia* was under stressful conditions caused by caffeine and ibuprofen respectively. Proteomic profile in *Daphnia magna* was also analyzed using a two-dimensional electrophoresis. As a result, we found that the expression patterns of protein profile in *Daphnia magna* are changed by toxicity of caffeine and ibuprofen. Based on this result, it was found that aquatic ecosystems exposed to pharmaceuticals can not be ignored.

**WP230 Uptake and Partitioning of TNT and RDX in Coastal Marine Organisms** M. Ballentine, University of Connecticut / Department of Marine Sciences; R. Smith, P. Vlahos, C. Tobias, University of Connecticut. The munitions constituents 2,4,6-trinitrotoluene (TNT) and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) are compounds commonly found in explosives and used widely at Department of Defense facilities, including coastal military installations. TNT and RDX and their derivatives are Environmental Protection Agency priority pollutants. Both have known toxicity with a variety of terrestrial and aquatic species and have been shown to persist in freshwater environments and soils. Less is known about TNT and RDXs fate and transport in marine systems. As part of a multi-scale marine ecosystem fate and transport study, we have measured degradation rates of TNT and RDX in seawater and characterized uptake of these compounds into several common coastal marine floral and faunal species. Time series of seawater and seawater plus fauna exposed to multiple elevated TNT and RDX concentrations were conducted in recirculating tanks. The selected organisms represent a cross section of common shallow water marine species found across multiple trophic levels. TNT and RDX concentrations (and associated breakdown products) in seawater and biomass were measured using a salting-out extraction with acetonitrile and GC/ECD. In seawater-only treatments, TNT loss from the water column was on the order of 0.16 mg L<sup>-1</sup> day<sup>-1</sup> to 0.2 mg L<sup>-1</sup> day<sup>-1</sup>, while RDX persisted at fairly constant concentrations for the duration of the incubation. In the presence of organisms, TNT removal from seawater was 2 to 3 times higher while RDX remained as persistent in the water as observed in the absence of fauna. TNT concentration increased rapidly in exposed organism tissue (within an hour) then slowly decreased over the remaining time period at a rate similar to that of water indicating a rapid equilibrium with water and no tendency to accumulate further. RDX concentrations in tissue also increased rapidly upon exposure but continued to accumulate linearly over a 10 day period. Bioconcentration factors (BCFs) were calculated for TNT, RDX, and their derivatives for each species. Analysis of trophic transfer of munitions using

stable isotope tracers is anticipated to be used to garner a better understanding of the fate and uptake of TNT and RDX in salt water systems in future follow-up experiments.

**WP231 A PBT Screening Tool for Allocation of Remedial Cleanup Costs at Superfund Sites** T. Biksey, WSP Environment & Energy / Director Risk Assessment, EHS Support, Inc. / Director Risk Assessment; A. Patz, N. Goulding, EHS Support, Inc.. An allocation of remedial cost screening tool was developed to assess Superfund constituents of potential concern (COPCs) identified in the remedial investigation phase of a Superfund project based on persistence, bioaccumulation, and toxic (PBT) potential. The purpose of this PBT approach was to accommodate the evaluation of potential COPCs related to historical discharge of process wastewater from proprietary chemical productions that did not have a CERCLA suite of laboratory analyses for comparison to the traditional CERCLA COPCs that were present in the sediments of the Superfund site. Using this approach, a preliminary allocation of remedial costs between the potentially responsible parties (PRPs) that may be liable for cleanup at a Superfund site can be performed to identify "de minimis" PRPs, and then focus additional allocation activities on those PRPs that are a significant contributor of the COPCs that are the risk-drivers for the remedial action. The PBT approach is consistent with regulatory guidance (e.g., EPA's PBT Profiler screening tool [www.pbtprofiler.net](http://www.pbtprofiler.net), EPA's Risk Assessment Guidance for Superfund [RAGS] and Guidelines for Ecological Risk Assessment) for assessment of potential PBTs in risk assessments, chemical classification, and regulation of chemicals. In addition, the PBT approach is consistent with the Gore Factors, specifically the third equitable factor, which examines the toxicity of the hazard and the belief that those parties involved who release COPCs that are more toxic are more responsible for the hazardous conditions created by their release. The PBT approach used for the COPCs assesses the PBT potential based on physical, chemical, and toxicological values, and then assess each COPC into one of three hazard buckets: high, medium, and low. The PBT approach is appropriate to assess the COPCs identified in a Superfund remedial investigation, especially when historic industrial wastewater discharges use proprietary materials in their processes. The approach is a transparent process that uses universally accepted EPA hazard identification methodologies, and is based on broadly adopted and globally applicable PBT databases and modeling software. An example of the PBT approach will be presented to demonstrate its applicability and technical basis as a remedial cost allocation screening tool to assist PRPs in negotiating their proportionate cleanup costs.

**WP232 Determining the Toxicity of Five Individual Polyaromatic Hydrocarbons to Early Lifestage Zebrafish Utilizing a Passive Dosing Approach** J. Butler, ExxonMobil Biomedical Sciences Inc. / Toxicology and Environmental Sciences; T. Parkerton, ExxonMobil Biomedical Sciences, Inc., Exxon Mobil Biomedical Sciences / Toxicology & Environmental Science; D. Letinski, ExxonMobil Biomedical Sciences, Inc.; K.R. Cooper, Rutgers, the State University of New Jersey / Department of Biochemistry and Microbiology. The target lipid model (TLM) has been used to derive water quality criteria that are intended to be protective of chronic effects posed by hydrocarbons. However, reliable experimental early life stage chronic toxicity data for fish are limited and further data are needed to confirm model predictions. Efforts are underway to develop a zebrafish embryotoxicity test guideline to reduce, refine and replace the use of vertebrates in animal testing. An adaptation of this method, which includes embryo lethal and sub-lethal developmental endpoints after a 5-d exposure as well as larval survival and growth endpoints during a subsequent 25-d test period, was utilized. Dose response exposures were conducted on 5 polyaromatic hydrocarbons with a Kow range of 3.88-6.06. To deliver well controlled exposure concentrations, a passive dosing system consisting of silicone o-rings loaded with test compound was employed. For all exposures, results indicated that embryonic lifestages were the most sensitive. Coagulation, lack of somite development, lack of tail bud detachment, no heart-beat and immobilization were lethal endpoints observed. Sub-lethal side effects such as pericardial and yolk sac edema as well as tail curvature were the most sensitive, and in many cases preceded observed mortality. NOECs were established for early (5d) and larval life stage (30d) exposures. Furthermore, results confirm that TLM predicted water quality criteria are protective of early life stage effects to all compounds tested. Further work is needed to determine the relative sensitivity of embryo versus larval chronic endpoints for additional hydrocarbons and test species.

**WP233 Prediction of Acute Aquatic Toxicity Endpoints of Pharmaceuticals by QSAR modeling** S. Moghaddam, Office of Pharmaceutical Science/Center for Drug Evaluation and Research /U.S. Food and Drug Administration; D. Quigley, Leadscape, Inc.; S. Page, OPS/CDER/FDA; R. Bloom, OPS/CDER/FDA / Senior Environmental Officer; N. Sadrieh, OPS/CDER/FDA. Quantitative structure-activity relationship (QSAR) models are used in drug development to help identify potential hazards. Generally, QSAR is most useful in those instances where little data are available to identify potential hazards important for appropriate regulatory decision making. ECOSAR, the ecological structure activity relationships program, has previously been shown to predict a variety of aquatic toxicity endpoints for pharmaceutical drugs. The ECOSAR equations for predicting the toxicity of a chemical compound combine an expert decision tree chemical class selection process with a computational method based on the correlation between the octanol-water partition coefficient (Kow) and acute aquatic toxicity. While ECOSAR is a commonly used model to make predictions about the ecotoxicity of chemicals, we have developed a battery of new ecotoxicity QSAR models using the Leadscape Predictive Data Miner software, with which we have previously developed a number of other models used for regulatory purposes. Our ecotoxicity QSAR models estimate the acute aquatic toxicity of pharmaceutical drugs based on chemical structural features in addition to physicochemical properties, such as ALogP, of both industrial chemicals and pharmaceutical drugs. These new models attempt to utilize common criteria used for the validation of QSARs, such as a defined response variable (Fish LC50 -96h), a partial regression algorithm using predictors derived from over 27,000 fingerprint features, a defined domain of applicability, mechanistic interpretation derived from underlying chemical structural features linked to a biological response, and appropriate measures of goodness-of-fit, robustness, and predictivity. Our models were validated against an external data set of values for drug LC50-96h toxicity results sourced from proprietary FDA environmental assessment submissions. Our proposed models present comprehensive acute ecotoxicity hazard identification for pharmaceuticals in that the results are linked to the relative contributions of toxifying and de-toxifying chemical features and properties.

**WP234 Toxicity of Untreated and Ozone-treated Oil Sands Process-Affected Water (OSPW) to Early Life Stages of the Fathead Minnow (*Pimephales promelas*)** Y. He, University of Saskatchewan / Toxicology Centre, University of Saskatchewan / PHD student; S. Patterson, M. Hecker, University of Saskatchewan / Toxicology Centre; M.G. Din, University of Alberta / Department of Civil and Environmental Engineering; J.W. Martin, University of Alberta, University of Alberta / Laboratory Medicine and Pathology, University of Alberta / Department of Lab Medicine and Pathology, University of Alberta / Department of Laboratory Medicine and Pathology, Division of Analytical and Environmental Chemistry, University of Alberta / Department of Public Health Science; J.P. Giesy, University of Saskatchewan / Biomedical Veterinary Sciences & Toxicology Centre, Michigan State University / Department of Animal Science, University of Saskatchewan / Toxicology Centre; S.B. Wiseman, University of Saskatchewan / Toxicology Centre, University of Saskatchewan / Post-Doctoral Fellow. Oil sands process-affected water (OSPW) is produced during the surface mining of oil sands in Alberta, Canada. The OSPW is stored in on-site tailings ponds due to a no-release policy. Ozonation has been proposed as an effective method for OSPW remediation by reducing levels of dissolved organic compounds, including naphthenic acids. Our previous *in vitro* studies demonstrated the endocrine disruptive toxicity of OSPW and the attenuation of these effects by ozonation. However, *in vivo* studies are needed to evaluate the effectiveness of ozonation in reducing the toxicity of OSPW and to ensure that ozonation does not impart toxicity upon OSPW. This study examined the effects of untreated and ozone-treated OSPW on early life stages of fathead minnows. Embryo mortality, spontaneous embryo movement, hatching success, and indicators of teratogenicity (hemorrhaging, pericardial edema, and spinal malformation) were examined. To elucidate the potential mechanism(s) of toxicity the concentration of reactive oxygen species (ROS) in the embryos was measured, and the abundances of transcripts of genes involved in the biotransformation of xenobiotics, response to oxidative stress, and apoptosis were quantified by real-time PCR. Compared to the control, exposure to OSPW decreased the embryo survival ( $43.75 \pm 7.12\%$ ), and exhibited a greater rate of spontaneous movement and greater incidences of premature hatching. Embryos exposed to OSPW had greater incidences of hemorrhage ( $50.00 \pm 3.40\%$ ), pericardial edema ( $56.25 \pm 7.12\%$ ), and spinal malformation ( $37.50 \pm 5.38\%$ ). Although

these effects were similar to those caused by dioxin-like compounds the abundance of transcripts of *Cyp1A1* was not significantly greater in embryos exposed to OSPW. Compared to the control, greater concentrations of ROS by  $1.68 \pm 0.11$ -fold and greater abundances of transcripts of *Cyp3a*, *Gst*, *Sod*, *Casp9*, and *ApopEn* by  $2.35 \pm 0.34$ ,  $2.15 \pm 0.26$ ,  $3.08 \pm 0.74$ ,  $3.26 \pm 0.57$ , and  $2.38 \pm 0.25$ -fold, respectively, indicated that exposure to OSPW might cause oxidative stress and apoptosis. Removal of organic constituents in OSPW by ozone significantly attenuated all of the adverse effects observed in the embryos exposed to OSPW. The results suggest that the organic fraction of OSPW adversely affects development of fathead minnow embryos through oxidative stress and apoptosis. Ozonation of OSPW attenuated this developmental toxicity.

**WP235 Depositional patterns of inorganics and polyaromatic compounds in precipitation in the Athabasca Oil Sands area of Alberta, Canada** D. Muir, Environment Canada / Aquatic Ecosystem Protection Research Division; X. Wang, Environment Canada / Aquatic Contaminants Research Division; J.L. Kirk, Environment Canada / Aquatic Contaminants Research Division; C. Teixeira, M. Duric, A. Sett, Environment Canada / Aquatic Contaminants Research Division; S. Backus, L. Davis, Environment Canada / Water Quality Monitoring and Surveillance Division; C. Mihele, G. Poole, Environment Canada / Air Quality Research Division. Atmospheric deposition has been identified as an important source of contaminants to the Athabasca River and its tributaries in the Oil Sands (OS) Region. Deposition has been investigated via measurements of winter snow pack, however, continuous measurements in precipitation throughout the year are lacking. To address this gap we installed organic and inorganic wet-only precipitation samplers at 3 locations within the OS development area north of Ft McMurray AB. Two of the collectors were located within 5 km of the OS bitumen upgrading facilities and the third was 10 km north of the nearest upgrader. Starting in December 2010, samples were collected monthly during 2011 and continued through 2012. Wet-only samples for polyaromatic compounds (PACs) were collected in a stainless steel funnel ( $0.212 \text{ m}^2$ ) into XAD-resin columns while samples for inorganics were collected as wet only bulk samples into a covered plastic bucket ( $0.03 \text{ m}^2$ ). Samples were analysed for total particulate organic carbon and other water chemistry parameters, 45 elements by ICP-MS, and 64 PACs by GC-MS. PAC profiles in precipitation were dominated by C1-C4 alkyl PACs, which represented 80-85% of the total PAC. Monthly fluxes of total PACs ranged from 10 to  $800 \text{ ug/m}^2$  and were highest in the winter months (January-March) during both 2011 and 2012. Total PAC fluxes averaged 3.5-fold lower at the site 10 km north than at the two near-field sites. Inorganics in precipitation were dominated by Al, Fe, K, Mg, Mn, Na, and Zn and contained ng/L concentrations of heavy metals As, Cd, Pb, and V. Average annual inorganic fluxes in precipitation ranged from  $3 \text{ ug/m}^2$  for Cd to  $1100 \text{ ug/m}^2$  for Zn and were highest during the January-March. Fluxes of metals and PACs in precipitation exceeded those reported for rural areas in Canada (e.g. in the Great Lakes region). Several metals including V, Mn, and As had much higher depositional fluxes than reported in urban and industrial areas in eastern North America while other elements were lower e.g. Zn and Cd.

**WP236 A weight-of-evidence approach to investigate the fate and effect of metals in the Athabasca River region** C.J. Rickwood, Natural Resources Canada / CANMET MMSL; N. Puttaswamy, S. Langley, Y. Thibault, Natural Resources Canada; C. Gagnon, Environment Canada; P. Huntsman-Mapila, Natural Resources Canada. There has been much debate as to the extent of the impact on the environment from the activities conducted in the development and processing of oil sands in the Athabasca River basin. Although most research has focused on the impact of organic contaminants such as hydrocarbons, more recently published work has highlighted metals as a potential environmental concern from oil sands processing. In response to this concern, a project was conducted using a weight-of-evidence approach to gain a better understanding of metal fate and effects within the Athabasca River region, specifically to address the potential linkages with oil sands development. This presentation outlines the use of multi-disciplinary techniques, including mineralogy, microbiology, chemistry and toxicology in understanding metal fate and effects in the Athabasca River. Results show that industrial inputs were clearly identified in the form of coke particles in sediment and snow samples and that the number of particles was highest at sites in the vicinity of oil sands processing. An elevation of Arsenic in downstream sites was observed but was clearly a function of



sediment particle size, DOC and microbial influences and not necessarily an anthropogenic input. Overall, metals analyzed were found to be primarily bound in the particulate phases. By using a weight-of-evidence approach we were able to investigate the various factors and mechanisms affecting metal mobilization. These types of mechanisms are important to understand if we are to gain further insight into the fate and effect of metals in this system.

**WP237 Changing sediment geochemistry in the Athabasca oil sands area: 1997-2011** D.H. de Boer, University of Saskatchewan; M. Evans, K. Janzen, Environment Canada. Oil sands development in the Athabasca River basin has led to substantial changes in land use and concerns for water and sediment quality. Cluster analysis of metal concentrations in sediments has resulted in the development of a metal signature potentially indicating human activity related to oil sands development in the basin. Metal concentrations in sediments collected from tributaries along the Athabasca and Mackenzie Rivers were normalized to Al to account for differences in the percentage of fines. Cluster analysis of the normalized concentrations indicated four distinct clusters. Clusters 1 and 3 were closely related and represented samples with low normalized metal concentrations. Clusters 2 and 4 were closely related and represented samples with high normalized metal concentrations, in particular Ti (cluster 2) and As, Ba, Co, Pb, Mn, Ni, K, V and Zn (cluster 4). From 1997 to 2002, the majority of tributary samples in the oil sands area belonged to clusters 1 and 3. However, starting in 2005 there was a shift in predominance to clusters 2 and 4. It is possible that oil sands operations mobilized sediment, resulting in a change in metal signature from clusters 1 and 3 to clusters 2 and 4 in the tributaries. These shifts in clusters appear to be associated with a change in sediment characteristics from one natural sediment source to another rather than with an increase in metal concentrations. Future research is planned to identify the possible causes such as rearrangement of channel networks leading to a shift in sediment sources and pathways, atmospheric pathways involving windblown dust and fly ash enriched in Ti and V, and changes in analytical procedures. Metal concentrations did not change significantly over the period of this study and thus do not appear to be an effective indicator of human activity in the Athabasca oil sand region as they. Sediment fingerprinting based on geochemical ratios therefore is a more useful tool to establish these changes.

**WP238 Temporal changes in PAHs and metals in the Athabasca River delta: 1997-2011** M. Evans, Environment Canada; D.H. de Boer, University of Saskatchewan; K. Janzen, Environment Canada. Concerns have been raised that the expanding oil sands operations are increasing metal and PAH loading to the Athabasca River delta through increased atmospheric emissions and disturbances to the landscape. We examine this issue by considering trends in metal and PAH concentrations and ratios in delta sediments over 1997-2011. There were no time trends in metal concentrations or in metal ratios suggesting that metals originating from atmospheric, eroded bank material, and water born sources had not changed appreciably over 1997-2011. PAHs were dominated by petrogenic sources with little evidence of a change in overall source over time. Parent PAH concentrations also did not change appreciably but total alkylated PAH concentrations increased slightly. Part of the increase could be attributed to laboratory methods where fewer compounds were measured at the beginning of the study. We contrast these trends with that observed in a sediment core from western Lake Athabasca and from Namur Lake, west of the development area. Namur Lake showed more evidence of combustion sources than western Lake Athabasca and its delta based on the ratios considered. This lake is impacted by events occurring in its small watershed with atmospheric deposition being the most probable source of contaminant input. Lake Athabasca receives the majority (90%) of its water flow from upstream of Fort McMurray which leads to strong dilution of local anthropogenic sources originating from oil sands development activities in the delta and western Lake Athabasca.

**WP239 Exposure of fish in the Athabasca and Slave Rivers to metals potentially derived from Oil sands operations** B. Tandler, E. Ohiozebau, A. Hill, University of Saskatchewan / School of Environment and Sustainability and Toxicology Center; G. Codling, University of Saskatchewan / Toxicology Centre; J. Giesy, University of Saskatchewan / Veterinary Biomedical Sciences; E. Kelly, Government of the Northwest Territories; P.V. Hodson, Queen's University / School of Environmental Studies; J. Short, DJWS Consulting LLC; P. Jones, University of Saskatchewan / School of Environment and Sustainability, University of Saskatchewan / School of Environment and

Sustainability and Toxicology Center. A large segment of the Alberta oil sand open-pit mining activities are adjacent to the Athabasca River. Aerial emissions and oil sands process-affected waters contain many different chemicals including polycyclic aromatic hydrocarbons (PAHs), naphthenic acids, and metals, some of which are persistent, toxic, mutagenic, and potentially carcinogenic. Concerns have been expressed about the potential environmental and human health effects of emissions by communities in the Athabasca River basins as well as by communities in the downstream Slave and Mackenzie river basins. These concerns have been exacerbated by uncertainties concerning the origin of these contaminants. The aim of this research was to describe the spatial and temporal distribution of metals in fish tissues from selected locations along the Athabasca and Slave Rivers. Target sample size for the project was 30 individuals of 5 species (Goldeye – *Hiodon alosoides*, Whitefish – *Coregonus clupeaformis*, Northern Pike – *Esox lucius*, Walleye – *Sander vitreus*, Burbot – *Lota lota*) at three locations on the Athabasca river and two locations on the Slave River. Fish were sampled in summer, fall, and winter of 2011. Concentrations of 20 metals were measured by fish tissues by ICP-MS. While most of the metals analysed showed little or no location associated variability, vanadium and thallium concentrations increased in fish collected downstream of oil sands operations. The latter two metals have been previously associated with discharges from petroleum-based activities. Statistically significant ( $p < 0.05$ ) increases in vanadium were observed at Fort Resolution and Fort Smith on the Slave River compared to sites on the Athabasca River. A steady increase in the concentration of thallium in fish muscle was noted at sites proceeding down the Athabasca/Slave system. How the observed metal concentration changes might be related to sources and to trophodynamics will be discussed.

**WP240 Modeling the transport and fate of polycyclic aromatic compounds in the Athabasca Oil Sands Region** A. Parajulee, University of Toronto Scarborough / Department of Physical and Environmental Sciences, University of Toronto; F. Wania, University of Toronto Scarborough / Department of Physical and Environmental Sciences. Development of the Canadian oil sands in Alberta, Canada, has generated much controversy due to its perceived environmental impacts, including the release of organic contaminants. The impacts of this release, particularly with respect to aquatic ecosystems, are not well characterized due to a paucity of high quality environmental monitoring data. The current study is a first attempt to characterize organic contaminant emissions and cycling in the Athabasca Oil Sands Region (AOSR) using a multimedia environmental fate model. Model-predicted concentrations for three representative polycyclic aromatic hydrocarbons (PAHs) in air, water, soil, and sediment are compared with environmental monitoring data to judge whether existing emissions data compiled either from the National Pollutant Release Inventory or from a recent Environmental Impact Assessment report are reasonable. Results suggest that the emissions scenarios provided by the two sources cannot explain concentrations of PAHs measured in various environmental media in the AOSR. Model simulations also indicate that direct emissions to air from oil sands operations may be a minor contributor of PAHs to the AOSR relative to indirect emissions resulting from quantities reported as disposal to tailings ponds. Furthermore, the indirect pathways from tailings ponds to freshwater via the atmosphere could be the most significant supply mechanism of oil sands PAHs to aquatic systems. Finally, the results of inverse modeling indicate that the emissions of PAHs required to account for observed environmental concentrations in the region exceed reported emissions by up to a few orders of magnitude. We also provide first emissions estimates for dibenzothiophene, also a product of oil sands upgrading.

**WP241 Biomonitoring 2.0 at Wood Buffalo National Park: a high-throughput genomics approach for comprehensive assessment of environmental change** M. Hajibabaei, University of Guelph / Integrative Biology, University of Guelph / Assistant Professor; J. Gibson, S. Shokralla, I. King, University of Guelph; D. Baird, University of New Brunswick. Biomonitoring seeks to describe and understand biodiversity at multiple ecological levels as a means to establish "biological early-warning systems" that can tell us when environmental stresses are reaching a critical point. Current practices are personnel-intensive and focus on a very limited subset of all species. Biomonitoring 2.0, uses next-generation DNA-sequencing technologies (NGS) and state-of-the-art computational analysis to assess biodiversity in any environmental setting. The first, large-scale test bed for this new system is Wood Buffalo National Park (WBNP), spanning northern Alberta and the Northwest Territories. This park is the ideal location for the



Biomonitoring 2.0 approach as it serves as a large-scale, preserved region directly adjacent to oil sands activities. Sample sites have been chosen in order to generate a base-line biodiversity information and evaluate sites that might be impacted by upstream Oil Sands extraction. At each site, samples are being collected across a 100m lateral wetland transect covering both aquatic and terrestrial habitats. These samples include soil cores, water samples, benthic samples, and terrestrial samples. Historical trend information, GIS data, and local physico-chemical analysis is also being compiled for each sample site. The samples collected are being used in NGS runs to generate over a million DNA sequences from the organisms of the park. Multiple target gene regions have been chosen for each kingdom of life, from bacteria, to plants, to fungi, to animals. Estimations of the biodiversity of each sample site is based on species-level DNA markers (DNA barcodes) and phylogenetic profiles of communities. By facilitating greater biodiversity discovery in a shorter amount of time, DNA-based NGS approaches will revolutionize biomonitoring programmes. The conventional, binary system (*i.e.*, present/absent, impacted/not impacted) will be replaced with more nuanced appraisals of the full spectrum of potential environmental impacts, based on the entire biota. Huge volumes of genomics information will allow the isolation of specific responses from underlying natural variability.

**WP242 A genomic fingerprint to test whether fish in the bitumen mining region of the Athabasca River are exposed to industry specific contaminants** J.P. Sherry, D. Simmons, T. Neheli, Environment Canada / Water Science & Technology Directorate; F. Gagne, Environment Canada, Centre St Laurent. Concern about the potential for adverse health effects on aquatic biota downstream of oil-sands operations means there is a pressing need to understand whether fish in the Athabasca River are exposed to industry specific contaminants – as opposed to naturally occurring constituents of bitumen. The health effects of complex mixtures of chemicals, such as the mixtures of PAHs, naphthenic acids, and heavy metals associated with oil sands operations result from exposure and the consequences of exposure. Complex mixtures typically induce a modulated pattern of gene expression in exposed fish. Once characterized and validated, elements of that response pattern can provide a diagnostic fingerprint of exposure to the mixture's constituents. We used FRAP-PCR to establish a gene expression signature in juvenile rainbow trout (*Oncorhynchus mykiss*) that were exposed to tailings pond (TP) water and Athabasca River water taken from upstream of oil-sands operations. We also used reverse phase high performance liquid chromatography tandem quadrupole time of flight mass spectrometry (RP-HPLC-QTOF) to explore differential protein expression signatures in the same fish. Our preliminary data showed that CYP1A mRNA was induced by TP water with a dose-response relationship. TP water also induced Vtg mRNA and protein (ELISA) in the TP exposed fish. We developed qPCR assays for each of the 20 mRNA transcripts selected for inclusion in the fingerprint. Comparison analysis of pooled proteomic profiles of plasmas sampled from the 10-d TP exposures identified a dose-response relationship for statistically significant molecules that were related to free radical scavenging. Pathways analysis of our data will enable us to explore links between the modulated transcripts/ proteins and to potential health effects.

**WP243 Aquatic Toxicity of naphthenic acids: modeling and alternative test methods** A. Redman, Hydroqual, Exxon Mobil Biomedical Sciences; T. Parkerton, D. Letinski, G. Bragin, R. Manning, T. Knarr, B. Kelly, C. Lee, ExxonMobil Biomedical Sciences, Inc. Oil sands process waters have complex chemistry that includes several organic and inorganic constituents. Naphthenic acids (NAs) represent an important class of compounds in these process waters. As a category, NAs are a mixture of cyclic, branched and linear organic compounds with oxygen-containing functional groups (*e.g.*, carboxylates, hydroxyls), whose exact composition can be quite variable. NAs can be toxic to aquatic life and this presentation will focus on the development of new toxicity data for select NAs that span the range of expected physico-chemical properties to support development of models and analytical methods for evaluating NA bioavailability. The aim of this work is to demonstrate proof of concept in simpler systems (*e.g.*, single chemical) before applying these methods to complex substances. For this work the Target Lipid Model (TLM) framework is being evaluated, since it is based on a large training dataset and has been used previously for the derivation of environmental quality guidelines. The TLM can be used to relate toxicity to the composition of NA mixtures, analogous to the application of the TLM to hydrocarbons. Initial applications of the TLM to organic acids were successful in relating physico-chemical properties to observed effect levels. In

addition to the modeling approach, passive sampling analytical methods are being evaluated in the toxicity testing in this study as a means of measuring the bioavailable concentrations of NA. Accumulation of test material, as individual compounds or as mixtures, on the passive sampling devices provides an integrated assessment of the exposure and can be related to critical effect concentrations (*e.g.*, LC50 or CBB). The results of initial applications of the passive sampling work to naphthenic acids were consistent with our previous work this technology to other complex substances.

**WP244 Toxicity of Oil Sands Naphthenic Acid Extracts to Early-Life Stages of Fathead Minnows** J.R. Marentette, / Psychology, Neuroscience and Behaviour, Environment Canada / Environment Canada, AEPRD; R.A. Frank, Environment Canada / AEPRD, Environment Canada / Dept. of Env. Biology & Toxicology, University of Guelph / Dept. of Env. Biology & Toxicology; L.M. Hewitt, J.V. Headley, Environment Canada; J.L. Parrott, Environment Canada / Environment Canada, National Water Research Institute / Environment Canada. Naphthenic acids (NAs) are major toxic constituents of oil sands process-affected water and thus a primary concern for the ultimate remediation of tailings ponds in Northern Alberta's oil sands regions. Fathead minnow embryo-larval survival, growth and behavior were studied after exposure to NA extract (NAE). NAs were extracted from fresh OSPW collected in 2009 from the Athabasca oil sands region. Fathead minnow embryos less than 24 hours old were reared in NAE ranging from 0 to 20 mg/L through to 15 days post-hatch (dph), in 1 L beakers for a 21-day exposure. Larvae were assessed at 8 and 15 dph for effects on growth and behavior (activity, feeding rate and startle response following a vibrational stimulus). NAE affected larval mortality but not hatching success at 20 mg/L (LC<sub>50</sub> = 15 mg/L), and few significant effects on larval size, activity, feeding rates or startle responses were noted. To more closely assess effects of NAE on hatching success and time to hatch, embryos less than 24 hours old were exposed to NAE ranging from 0 to 80 mg/L until hatching for a 5+ day test in 24-well tissue culture plates. Length at hatch was measured, as were additional embryonic endpoints of heartbeat and flexure rates at ages 1 to 3 days post-fertilization. Heartbeat reflects cardiovascular development, and embryonic flexures serve in part to distribute dissolved oxygen and hatching enzymes in perivitelline fluid, facilitating development. NAE reduced heartbeat rate, tended to affect flexure frequency, extended time to hatch, and ultimately decreased hatching success (LC<sub>50</sub> = 14 mg/L), but showed no effects on hatch length, in this fish embryo toxicity test. Possible sources for the variation in effects on hatching success between the two types of early-life stage toxicity tests, as well as comparisons to commercial NA mixtures, are explored in currently ongoing studies.

**WP245 Naphthenic acids are toxic, and disrupt development and metabolic processes in anuran tadpoles** S. Melvin, Canadian Rivers Institute, University of New Brunswick / Canadian Rivers Institute; C. Lancot, Centre for Advanced Research in Environmental Genomics, University of Ottawa / Biology; P. Craig, T.W. Moon, University of Ottawa / Biology; V.L. Trudeau, University of Ottawa / Advanced Research in Environmental Genomics. Naphthenic acids (NAs) are a diverse group of naturally occurring low molecular weight constituents of petroleum with a wide range of commercial applications. Release of NAs in Oil Sands Processed Water to the aquatic environment may represent an emerging threat to frog populations. For a technical grade mixture of NAs, we determined LC50 values of 4.8 and 3.0 mg/L for *Lithobates sylvaticus* tadpoles at Gosner stage (GS) 28 and GS36, respectively. Similarly, LC50 estimates for embryos were 4.1 mg/L for 72-h exposure in *Lithobates pipiens* and 4.1, 3.5, and 3.0 mg/L for 24-, 48-, and 72-h exposure in *Silurana tropicalis*. Our results demonstrate that embryos of *L. pipiens* and *S. tropicalis* exposed to 2-4 mg/L NAs exhibited reductions (32% and 25%, respectively) in growth and development upon hatching. Increased incidences of deformities were also observed in *L. pipiens* tadpoles exposed to NAs at these concentrations. *L. pipiens* tadpoles exposed to 1 and 2 mg/L NAs (similar to levels in groundwater samples) from GS26-42 (~75d) suffered decreased growth and development, significantly reduced glycogen stores, and increased levels of triglycerides. These results indicate that concentrations of NAs similar to those in the Alberta oil sands region disrupt energy metabolism and the hepatic glycolytic pathway. Reductions in energy stores may explain decreased growth, and smaller tadpoles are known to have reduced survival and reproductive potential as adults. Disruption of the thyroid status of developing Anura is one further hypothesis that needs to be tested as a potential mechanism whereby low concentrations of NAs upset metamorphosis.

**WP246 Application of Ion-Mobility Separation with High-Resolution MS for the Analysis of Oil Sands Process-Affected Water Organic Acid Content** L.A. Perez-Estrada, University of Alberta / Civil and Environmental Engineering; J.W. Martin, Trent University / Division of Analytical and Environmental Toxicology; M. Gamal El-Din, University of Alberta / University of Alberta. Large amounts of water are required to extract bitumen from the oil sands in northern Alberta. The generated tailings water, called oil sands process-affected water (OSPW), contains a complex mixture of organic and inorganic compounds that have been proven to be toxic to microbes, fish and mammals. Due to the high toxicity and incomplete characterization of OSPW, a zero-discharge policy has been implemented. The result has been the creation of tailing lakes that are estimated to cover an area of 130 square kilometers. Naphthenic acids (NAs;  $C_nH_{2n+2}O_2$ ) are a group of organic acids in OSPW that are thought to contribute to the toxicity of this water. NAs are a group of carboxylic acids that contain from 7 to 22 carbons and could be acyclic or have from 1 to 6 pentyl or hexyl rings, or a combination of both. The quantification of NAs traditionally has been done using spectroscopy techniques, such as FT-IR, but recently has moved to the use of (ultra-) high-resolution mass spectrometry, which has improved the level of selectivity. In this study we are reporting the first results from a two-dimensional separation technique using ultra pressure liquid chromatography (UPLC) coupled to an ion-mobility separation (IMS) with TOF-MS (Waters Synapt G2) detection. Chromatographic separation was done on a BEH-phenyl column with gradient elution, and an electrospray source was operated in negative mode to focus the analysis on organic acids. IMS wave velocity was set at 900 m/s, and the TOF achieved a resolution >40,000 with a "W" beam shape. Separation of the OSPW organic acid content showed three different groups of compounds with different retention times and similar drift times. Compounds within the group with shorter retention time were identified as oxidized-NAs. The other two groups have almost the same retention time but different drift times. Compounds in the low mobility group were identified as NAs, whereas compounds within the high mobility group were characterized as  $O_2$  species containing sulphur and nitrogen. Using retention time information, molecular shape relationships (IMS) and fragmentation profiles, it was possible to elucidate the general molecular structures of characteristic compounds in each group. These results showed that IMS is a very effective addition to the HRMS characterization of OSPW organic acids.

**WP247 Comprehensive oil sands process affected water analysis by liquid chromatography coupled to an LTQ Orbitrap mass spectrometer** A. dos Santos Pereira, Department of Lab Medicine and Pathology, Faculty of Medicine and Dentistry, University of Alberta. / Division of Analytical and Environmental Toxicology; S. Bhattacharjee, Faculty of Engineering, University of Alberta. / Department of Mechanical Engineering; J.W. Martin, Faculty of Medicine and Dentistry, University of Alberta / Division of Analytical and Environmental Toxicology, Department of Lab Medicine and Pathology. In the surface mining oil sands industry, toxic oil sands process affected water (sm-OSPW) is a by-product of extracting bitumen from oil sands. Despite heavy recycling of OSPW tailings ponds continue to grow. By 2009, tailings ponds covered approximately 130 km<sup>2</sup> and contained as much as a billion liters of sm-OSPW. The toxicity of OSPW from in situ mining (is-OSPW) is not known, and much less is known about its chemical composition, relative to sm-OSPW, but an understanding of the polar organic compounds in is-OSPW is an important consideration for steam generation (since the water is recycled) and also for controlling corrosion. In the present study, a highly resolved and sensitive method was established for rapid (20 min) and reliable characterization of OSPW by linking HPLC to a hybrid LTQ-Orbitrap mass spectrometer. Qualitative analysis was carried out in negative and positive ionization modes using electrospray. Mass spectral data was consistently > 100,000 resolution in full scan mode, even when acquiring with the HPLC. Optimal chromatography was achieved with a 2.5  $\mu$ m particle C18 HPLC column, allowing discrimination of various isomeric species that could not be distinguished by simple infusion including a large group of "non-naphthenic acid"  $O_2$  species. The method also permitted easy visualization of the homologues series of classic naphthenic acids, according to Z and carbon numbers, and simultaneous characterization of thousands of compounds containing carbon, hydrogen, oxygen, sulfur and nitrogen. is-OSPW was similar to sm-OSPW in terms of the major species present, but careful analysis of the homologues and isomers revealed many important differences. The features of the present method also make it a very attractive tool for environmental monitoring programs.

**WP248 Impact of the geochemical changes in tailing ponds on the release of hydrogen sulfide gas** A. Wijdeveld, W.v. Kesteren, DELTARES; T. Siddique, University of Alberta / Soil chemistry-environmental microbiology. The high sulfur content of the tailings and the sometimes additional source of sulfate (by the addition of gypsum) in the tailing ponds can cause the production and release of hydrogen sulfide (H<sub>2</sub>S) gas. The condition under which hydrogen sulfide gas is formed (or degraded) depends on the geochemical conditions in the pond. We developed a model in which the production, transport and release of hydrogen sulfide is related to the geochemical status of the pond. In this model we especially look into the role of iron on the (im)mobilization of sulfides. We also have established the formation rate of H<sub>2</sub>S at different depths in the pond, resulting to sources and sinks zones in the sediment. By also considering the gas production rate of methane and carbon dioxide (including the buffering of carbon dioxide by the dissolution as bicarbonate) we predicted the average annual release of H<sub>2</sub>S gas over time (1 – 100 years). Understanding the likely release rate of H<sub>2</sub>S over time helps in evaluating the environmental risk of the H<sub>2</sub>S release. Also, the model included the interaction of H<sub>2</sub>S with the dissolved and solid fraction and the model takes into account chemical reactions in and between different phases (like the weathering of gypsum, the precipitation of iron sulfide minerals or the redox depended oxidation of sulfite towards sulfate). Therefore the impact of possible H<sub>2</sub>S emission reducing measures can be evaluated and ranked based on efficiency and timescale.

**WP249 An evaluation of the sensitivity of lab-reared and field-collected *Hyalella azteca* from urban and ag areas to the pyrethroid insecticide cypermethrin** S. Clark, S. Ogle, A. Gantner, Pacific EcoRisk; J.M. Giddings, Compliance Services International; G. Mitchell, FMC Corporation; A.C. Barefoot, Du Pont Crop Protection / Crop Protection Products; D. Tessier, DuPont Crop Protection; M.G. Dobbs, Bayer Crop Science, Bayer CropScience / Ecotoxicology Group; P. Hendley, Syngenta Crop Protection, LLC. / Senior Syngenta Fellow, Syngenta Crop Protection, LLC. / Product Safety; K.S. Henry, Syngenta Crop Protection, Inc. / Ecological Sciences. *Hyalella azteca* are epibenthic macroinvertebrates that are easy to culture and are widely used for sediment toxicity studies, and have seen recent use as in water column toxicity testing. For example, *H. azteca* have recently seen considerable use in Clean Water Act (CWA) related water toxicity testing in California, as these organisms are more sensitive to pyrethroid insecticides than other standard invertebrate toxicity test species (e.g., *Ceriodaphnia dubia*). Resident *H. azteca* are known to occur in some sediments containing pyrethroid pesticides at concentrations that are toxic to *H. azteca* in lab tests. This suggests potential significant differences in the sensitivity of lab-reared and resident field populations of *H. azteca* to pyrethroids. The goal of this study was to determine the sensitivity of various *H. azteca* populations (including field populations) to the pyrethroid cypermethrin. *H. azteca* were collected from 3 Northern California sites with historically robust populations of *H. azteca* and which receive urban and/or agricultural runoff: Pleasant Grove Creek (PGC), Bear Creek (BC), and Laguna Creek (LC). Lab-reared *H. azteca* were obtained from a widely-used commercial vendor, and were 7-14 days old and within 2 days of age when initiating testing. Since the field organisms could not be aged, they were sieved to obtain a size close to that of the lab organisms. The *H. azteca* were exposed to cypermethrin at nominal concentrations of 0.28, 0.83, 2.5, 7.5, and 22.5 ng/L for 96 hours. The LC50 for the 'lab' organisms ranged from 5.8 to 12.1 ng/L; there was complete mortality at the 22.5 ng/L treatment. The LC50's for the field organisms were 13 ng/L (BC) and >22.5 ng/L (for PGC and LC); at the 22.5 ng/L treatment, there was complete mortality for the BC organisms and ≥90% survival for the PGC and LC organisms. These results demonstrate that lab-reared *H. azteca* can be more sensitive to cypermethrin than field-collected organisms. In order to determine how common such differences in sensitivity are, testing is underway with *H. azteca* collected from additional sites influenced by urban and agricultural runoff, as well as from remote sites that are not expected to receive urban and/or agricultural runoff.

**WP250 Automated online solid phase extraction LC-MS/MS for the determination of endosulfan isomers and their metabolite in water samples** N. Quinete, Florida International University (FIU) / Southeast Environmental Research Center; P. Gardinali, Florida International University (FIU) / Southeast Environmental Research Center (SERC) and Department of Chemistry and Biochemistry. Over the last 30 years, endosulfan has received considerable attention and has been the subject of a number



of international regulations and action plans worldwide. In South Florida insecticide application is nearly double the national average representing a major concern. The presence of endosulfan isomers and endosulfan sulfate in surface water in this region has been shown to exceed in some locations the U.S. EPA's chronic water quality criteria of  $\alpha$ - and  $\beta$ -endosulfan in fresh waters (56 ng/L). Endosulfan sulfate has been the major endosulfan metabolite detected in surface water specially along the C-111 and C-111E canals located in the Everglades area, which drain the Homestead agricultural area and discharge to either Florida Bay or Biscayne Bay, both of which are critical wildlife habitats. A selective, sensitive and fast online SPE coupled with a liquid chromatography-tandem mass spectrometry method for the determination of endosulfan isomers and endosulfan sulfate in water samples at low ppt levels was developed and validated. An atmospheric pressure chemical ionization (APCI) source operating in negative ion mode was chosen because it produced cleaner and more reproducible spectra of the studied compounds with no adduct ion formation. The method was able to separate and quantify endosulfan compounds within a 24 min run using acetonitrile and water as mobile phases. The automated online SPE clean-up and pre-concentration step was performed using only 20 mL of untreated water samples prior to LC-MS/MS analysis. With this approach, all compounds were first trapped on a SPE column and then back flushed onto a reverse phase LC column to MS/MS analysis. Analyte quantitation from the selected reaction monitoring (SRM) experiments relied also on the use of a deuterated ( $d_4$ )  $\beta$ -endosulfan as internal standard. The final method presented a linear range from 5 to 2000 ng/L and method detection limits (MDLs) of 3 ng/L for endosulfan sulfate, 10 ng/L for  $\alpha$ -endosulfan and 7 ng/L for  $\beta$ -endosulfan were achieved. Total analyte recoveries in tap water were performed in triplicate at three different concentration levels (MDL, 5xMDL and 10xMDL) and ranged from 88 to 140%. Matrix interferences were also evaluated. The developed methodology was applied to monitor the presence and assess the potential transport of endosulfan in the Loveland Slough watershed, an area adjacent to Everglades National Park showing long-term contamination with endosulfans.

**WP251 Comparison of Persistent Organic Pollutant Concentrations in Fish Collected from Western National Parks to Fish Consumption Guidelines** J.E. Schrlau, Oregon State University / Chemistry; C. Schreck, M. Kent, Oregon State University / Department of Fisheries and Wildlife; T. Blett, C. Flannagan, National Park Service; S. Massey Simonich, Oregon State University / Department of Chemistry. Concentrations of persistent organic pollutants (POPs) in fish collected from remote, high-elevation ecosystems of U.S. national parks (NP) are a potential ecological and human health concern. Based on the findings of the Western Airborne Contaminants Assessment Project (WACAP), the contaminant load in fish was further investigated in a total of 53 fish from five NPs including Rocky Mountain NP, Great Sand Dunes National Monument, Yosemite NP, Lassen Volcanic NP, and Wrangell-St. Elias NP & Preserve. The pesticides that were measured in >75% of the fish samples included p,p'-dichlorodiphenylethane (p,p'-DDE), hexachlorobenzene (HCB), chlorpyrifos, mirex,  $\Sigma$ chlordanes (*cis*-chlordane, *trans*-chlordane, *cis*-nonachlor, and *trans*-nonachlor), dacthal and endosulfan sulfate. The highest p,p'-DDE, HCB, chlorpyrifos, and mirex concentrations were measured at Mildred Lake in Yosemite, Tanada Lake in Wrangell-St. Elias, Ysilon Lake in Rocky, and Summit Lake in Great Sand Dunes, respectively. Alpha-hexachlorocyclohexane (HCH) was measured in 70% of the fish samples, with significantly higher concentrations at both Tanada and Copper Lake in Wrangell-St. Elias. Gamma-HCH was measured in 5% of the fish samples, with significantly higher concentrations in Copper Lake and Spirit Lake in Rocky. No fish SOC concentration in any park exceeded the threshold for recreational fish consumption. However, all lakes in this study, except for Dream Lake in ROMO, Lone Pine Lake in Rocky, and Ypsilon Lake in Rocky, had dieldrin concentrations in individual fish that exceeded the guideline for subsistence fish consumption. All POP concentrations were below the wildlife health threshold for mink and river otter. The  $\Sigma$ chlordanes wildlife health threshold for kingfisher (4.5 ng/g) was exceeded in one fish at Nanita Lake in Rocky and also p,p'-DDE (20 ng/g) in several fish at Mildred Lake in Yosemite.

**WP252 Development of a Pesticide Emissions and Exposure Model in Air (PEEMA) for Evaluation of Inhalation Exposure in EPA Risk Assessments** G. Rothman, US EPA; C. Peck, J. Decant, F. Khan, E. Odenkirchen, US EPA Office of Pesticide Programs. Under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), the U.S. Environmental

Protection Agency (EPA) evaluates ecological risks to non-target terrestrial and aquatic organisms from potential pesticide exposure pathways. To address inhalation risks for birds and mammals, EPA has implemented the Screening Tool for Inhalation Risk (STIR) to identify related data requirements needed for the risk assessment. In addition, at the recommendation of FIFRA Scientific Advisory Panel, the EPA is now developing the Tier I Pesticide Emissions and Exposure Model in Air (PEEMA). PEEMA will predict pesticide volatilization, emissions, and resulting air concentrations from crops and soil surfaces as well as the droplet-phase concentration in air resulting from aerial and ground pesticide sprays. The volatility scheme of PEEMA will consist of two components: 1) prediction of emissions from treated soil and crops to the air based upon physical chemical properties using available peer-reviewed data and 2) estimation of exposure air concentrations incorporating the estimated emissions using an atmospheric dispersion model. The droplet-phase scheme of PEEMA will utilize factors such as spray droplet size distribution and pesticide application rate to estimate the respirable air concentration for mammals and birds. PEEMA will provide estimated exposure concentrations of airborne vapor-phase and droplet-phase pesticides on the treated field, and the downwind distance from treated fields to the boundary where the level of concern is no longer exceeded. This paper will present the EPA's preliminary evaluation of the PEEMA model.

**WP253 Mapping of Depositional and Non-Depositional Areas in Salinas California Streams with Concurrent Pyrethroid and Benthic Macroinvertebrate Assessments** L.W. Hall, University of Maryland / Wye Research & Education Center; R. Anderson, University of Maryland; K. William, University of Maryland / Wye Research & Education Center. This study used sediment mapping to determine the spatial extent of depositional and non-depositional areas in the wetted stream bed of four urban streams in Salinas, California. After the stream mapping was completed, eight pyrethroids were analytically measured from randomly selected sites in 12 depositional and 12 non-depositional areas in the four Salinas streams. Benthic macroinvertebrate samples were collected and identified and physical habitat was measured from depositional and non-depositional areas where pyrethroids were measured. Based on a random sampling design, 24% of the 96 sediment sampling sites in the Salinas streams were classified as predominately depositional areas. Mean total pyrethroid concentrations were approximately 2x to 61x times higher in depositional areas of the Salinas streams when compared to non-depositional areas. Physical habitat scores from the 12 depositional and 12 non-depositional areas in the Salinas stream sites were similar and extremely low compared with other California streams thus demonstrating that impaired physical habitat is a critical stressor in these streams. Approximately 6,300 individual macroinvertebrates were picked and identified from 70 taxa from the 24 Salinas stream sites. The most dominant taxa collected were all considered tolerant of environmental stressors and dominant taxa from both depositional and non-deposition areas were similar. Ten different benthic metrics for the Salinas streams were similar for the depositional areas and non-depositional areas. Measured pyrethroid concentrations in depositional areas consistently exceeded laboratory based toxicity thresholds. Measured concentrations in non-depositional areas were consistently lower than laboratory based toxicity thresholds. These results suggest that factors other than pyrethroids are responsible for impacting resident benthic communities in these urban Salinas streams.

**WP254 Organochlorine Pesticides in Quail from the Rolling Plains** C.E. Baxter, Texas Tech University / Environmental Toxicology, Texas Tech University; R.J. Kendall, Texas Tech University / The Institute of environmental and human health. In the past few years quail populations in the Rolling Plains region in northwest Texas and western Oklahoma have experienced an idiopathic decline. Numerous phenomena have been suggested as the cause of this decline including drought, habitat loss, disease, heavy metals, and pesticides, but not one factor has so far been proven to be exceptionally detrimental to quail. Organochlorine pesticides are a subject of particular interest because they are known to cause toxic effects in avian individuals. Additionally, the agricultural nature of the Rolling Plains allows for frequent pesticide exposures. In order to evaluate the possible role of organochlorine pesticides in this quail decline, bobwhite quail (*Colinus virginianus*) and scaled quail (*Callipepla squamata*) tissue samples were collected during late summer into fall 2011 from sites across the Rolling Plains. Tissues from the leg muscles (n=157) were analyzed for twenty organochlorine pesticides using the QuEChERS method of extraction followed by GC-ECD/ECD



analysis. Pesticide levels were examined for significance based on location, age and sex. There were not enough scaled quail samples to test for significance between species. Found pesticide concentrations were also compared to those known to elicit toxic effects in birds in order to provide a general risk assessment. As a further step, leg muscle tissues from bobwhite quail bred in captivity were analyzed as well and the results were compared to those from wild birds in order to examine relative differences in exposure.

**WP255 Patterns in the toxicity of synthetic pyrethroids to aquatic species**

J.M. Giddings, Compliance Services International; M.G. Dobbs, Bayer Crop Science, Bayer CropScience / Ecotoxicology Group; K.S. Henry, Syngenta Crop Protection, Inc. / Ecological Sciences. Synthetic pyrethroid insecticides are an important group of chemicals in the urban aquatic environment. The Pyrethroid Working Group (PWG) has worked for several years to obtain, evaluate, and compile all available relevant, reliable data on the toxicity of synthetic pyrethroids to aquatic organisms. The PWG aquatic toxicity database now contains endpoints and supporting information from more than 700 open literature publications and registrant-sponsored toxicity study reports on 8 pyrethroids and nearly 350 species. From the available data, objective criteria were applied to select the most reliable and representative toxicity values for each species. Acute Species Sensitivity Distributions (SSDs) were derived for each pyrethroid for fish, crustaceans, insects, and mollusks, where data permit. The 5th percentile (HC5) and the median or 50th percentile (HC50), points that are often used as regulatory benchmarks and assessment endpoints, were calculated for each SSD. The data were further explored to characterize the positions of standard test species in the SSDs. The relative positions of many species are similar on the SSDs of different pyrethroids. For example, the most sensitive invertebrates are invariably amphipods (e.g., *Hyalella azteca*) and mysids. *Daphnia magna*, which is near the low end of SSDs for many pesticides such as organophosphates, is typically in the mid-range of pyrethroid SSDs for crustaceans. The Eastern oyster, *Crassostrea virginica*, is, like other mollusks, near the upper end of pyrethroid SSDs. Such generalizations are useful for interpreting pyrethroid toxicity data for standard test species and for characterizing risk to aquatic communities.

**WP256 Surface water contamination within residential-use areas with organo-nitrogen/organo-phosphate pesticides**

J. Wu, J. Lu, University of Florida/IFAS Indian River Research and Education Center; P. Wilson, University of Florida / IFAS / IRREC- Soil & Water Science; Y. Lin, University of Florida/IFAS Indian River Research and Education Center. The occurrence of organo-nitrogen (ON) and organo-phosphate (OP) pesticides in surface water has attracted public attention due to their potential risks to ecosystem and human health. A weekly monitoring study was conducted from September 2008 to July 2010 to investigate the occurrence of ON and OP pesticides, including desisopropyl atrazine, desethyl atrazine, simazine, atrazine, diazinon, chlorpyrifos-methyl, bromacil, malathion, metolachlor, chlorpyrifos-ethyl, napropamide, and norflurazon. diazinon, chlorpyrifos-methyl, malathion, metolachlor, and chlorpyrifos-ethyl were never detected at any of the eight sampling sites during the monitored period. Atrazine was the most frequently detected pesticide, with concentrations ranging from 0.11-11.08  $\mu\text{g L}^{-1}$ . Detection frequency for atrazine ranged from 8.3 to 91.7% (most >45%) for six residential canals, and from 95.8 to 96.9% for two lakes. Desethyl atrazine was detected at seven sampling sites with concentrations ranging from 0.11-0.42  $\mu\text{g L}^{-1}$ , and detection rates ranging from 2.1 to 12.5% of samples collected from the canals and from 18.8 to 28.1% for the lakes. Desisopropyl atrazine was only detected in two lakes, with concentrations ranging from 0.05-0.16  $\mu\text{g L}^{-1}$ , and detection rates ranging from 4.2 to 7.3%. Simazine was detected in 3 canals and one lake, with concentrations ranging from 0.11-0.27  $\mu\text{g L}^{-1}$ , and percent detection rates ranging from 1.0 to 9.4%. Bromacil, napropamide, and norflurazon were detected at three sampling sites with very low percent detection (1.0%) and concentrations ranging from 0.34-0.37, 0.37-0.78, and 0.4-0.6  $\mu\text{g L}^{-1}$ , respectively. Chlorpyrifos-ethyl, chlorpyrifos-methyl, diazinon, malathion, and metolachlor were never detected at any of the sites.

**WP257 Toxicity in California Surface Waters: 2001-2010**

B. Anderson, University of California – Davis / Department of Environmental Toxicology; D. Markiewicz, University of California; J. Hunt, University of California – Davis / Department of Environmental Toxicology; B.M. Phillips, University of California – Davis / Environmental Toxicology, University of California-Davis / Granite Canyon; K. Larsen, California State Water

Resources Control Board. Toxicity testing has been used to assess effluent and surface water quality in California since the mid-1980s. A recent Surface Water Ambient Monitoring Program (SWAMP) report summarizes the findings of monitoring conducted between 2001 and 2010. SWAMP monitoring has demonstrated that surface water toxicity occurs in all regions of the state, but that a greater number of toxic samples have been detected in the valleys and along the coast, the areas of greatest human activity. Greater than 50% of fresh water sediment and water samples and greater than 45% of marine sediment samples demonstrated some degree of toxicity in monitoring conducted between 2001 and 2010. While greater water toxicity was been observed in agricultural sites relative to urban sites, there was no difference in sediment toxicity between urban and agricultural sites. Correlation analyses and toxicity identification evaluations (TIEs) were used to determine the likely causes of surface water toxicity. TIEs conducted in water samples suggest that toxicity to invertebrate test species (e.g., cladocera and amphipods) was most often caused by pesticides (e.g., diazinon and chlorpyrifos). More recent studies also show that pyrethroid pesticides play a role in water column toxicity to amphipods. Correlation analyses corroborate the TIE findings. TIEs conducted with sediments show that sediment toxicity is caused by pyrethroid and organophosphate pesticides in freshwater habitats, and pyrethroids have also been shown to be causing toxicity in marine sediments. Triad assessments linking chemistry, toxicity, and macroinvertebrate community surveys have demonstrated that pesticide toxicity in the lower reaches of several California watersheds is linked to impacts on resident aquatic macroinvertebrate communities. Additional research has suggested that habitat also plays an important role in the distribution of stream macroinvertebrates. Because evidence from SWAMP monitoring has demonstrated that pesticides are a primary cause of surface water toxicity in California, management initiatives are being implemented to reduce pesticide loading throughout the State's urban and agricultural watersheds.

**WP258 Differences in US and EU Policies for Regulation of Chemicals**

J.A. Fernandez II, Arizona State University; R. Halden, Arizona State University / Biodesign Institute; H. Sanderson, Aarhus University / Environmental Science, National Environmental Research / Department of Policy Analysis. This study examined differences in policies between the United States and the European Union concerning the regulation of pesticides by example of the triazine compound, atrazine (2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine). Utilizing the same input data, atrazine is treated differently between two separate regions of the world, based upon safety perspectives and policy regulation paradigms. In 2007, atrazine was the second most widely used pesticide in the United States. On the other hand, it has been banned from use in the European Union since 2004 for reasons discussed in this presentation. The research methodology involved a literature review and policy analysis using online scientific databases, governmental agency websites and their databases to gather information and uncover the differences in policy decision-making of the United States and the European Union. Obtained data show that the United States bases decision making in agricultural pesticide policy first on maximum agronomic crop yield and efficiency and then on environmental protection and human health, by using a four-step risk assessment paradigm. In contrast, the European Union bases its policy making primarily on the protection of the environment and human health and then, secondarily, on agricultural productivity and efficiency using their risk assessment protocol, known as The Impact Assessment for a Regulation, formerly Council Directive 91/414/EEC or "The Directive". The European Union's stricter and more cautious approach can be understood as a form of the precautionary principle, which states, in short to be "rather safe than sorry". It can be inferred from this research that when it comes to policy making, specifically in reference to the regulation of agricultural pesticides, the United States and European Union have opposing views that ultimately result in differing policies and regulatory statutes.

**WP259 A Comparison of Passive Sampling Devices for Dissolved and Nanoform Silver in the Aquatic Environment**

J. Fischer, E. Hoque, Trent University / Environmental & Resource Studies; C.D. Metcalfe, Trent University / Environmental & Resource Studies, Trent University / Department of Environmental & Resource Studies; H. Hintelmann, Trent University / Chemistry. The use of silver nanoparticles in consumer products, industrial applications and health care technology is rapidly growing, due to their high catalytic activity and strong bactericidal properties. With such widespread usage, their inadvertent release into the environment is unavoidable. Since

the majority of silver nanoparticles in consumer products enter sewer systems and then are potentially released through waste-water treatment plants, assessments of environmental impacts are primarily focused on exposures to silver in water. It has been shown that ng/L concentrations of silver nanoparticles and dissolved silver can inhibit the growth of bacterial communities, and may be toxic to invertebrates and fish, but there are no currently available analytical techniques that can detect these low concentrations in aquatic matrices. To move forward with an environmental risk assessment of silver nanoparticles, it is essential to develop pre-concentration techniques that can be used to quantify trace concentrations of silver in water in nanoform and in dissolved form. We developed and tested three types of passive samplers for the selective detection of dissolved silver and silver nanoparticles in aquatic environments, including Diffusive Gradient in Thin Film (DGT) probes using chelex and thiol resins, Chemcatcher devices using chelating and anionic sorption disks, and surfaces impregnated with carboxylic and amine functionalized carbon nanotubes. Experiments in the laboratory compared the uptake of citrate- and PVP-capped nanoparticles, and dissolved silver. Studies are currently underway to field test the most promising passive sampling devices in the Grand River watershed, which receives discharges from 26 waste-water treatment plants in the Kitchener-Waterloo region of Ontario and in mesocosms deployed in a lake in the Experimental Lakes Area of Ontario as part of the Lake Ecosystem Nanosilver (LENS) project. Optimized techniques with passive samplers will provide estimates of time-averaged concentrations in water and will pre-concentrate the analytes to levels that can be detected using ICP-MS analysis. Results from laboratory experiments and field measurements will be presented and compared. This work is an important step for risk assessment of silver nanoparticles in the environment.

**WP260 Carbon nanotubes as a sorbent for solid phase extraction and preconcentration of silver nanoparticles prior to analysis by ICP-MS** E. Hoque, J. Fischer, Trent University / Environmental & Resource Studies; H. Hintelmann, Trent University / Chemistry; C.D. Metcalfe, Trent University / Environmental & Resource Studies, Trent University / Department of Environmental & Resource Studies. Because of its antibacterial properties, silver nanoparticles or nanosilver (nAg) are widely used in consumer products, such as textiles, personal care products, food containers, laundry detergents and home appliances. It is likely that nAg is discharged with wastewater from municipal and industrial sources into aquatic ecosystems. Hence, it is important to develop analytical methods to extract, preconcentrate and quantify nAg in samples of water and wastewater. In this study, we used carbon nanotubes (CNT) as a sorbent for solid phase extraction (SPE) to extract nAg, and then analyzed the extracts by inductively coupled plasma mass spectrometry (ICP-MS). The CNT surface has a strong interaction with other molecules or atoms because of its hexagonal arrays of carbon atoms in graphene sheet structure. Different types of CNTs, including NH<sub>2</sub> and COOH functionalized material are being investigated as the solid phase. Preliminary data using a CNT-NH<sub>2</sub> based SPE system shows that this sorbent can extract both polyvinylpyrrolidone (PVP) and citrate capped nAg (50 nm) and dissolved Ag (dAg) from water using a 50 mL sample volume (10 ng/mL) and 5 mL of 5% HNO<sub>3</sub> for elution. Under these conditions, the concentration enhancement factor and % recovery are  $4.7 \pm 0.3$  (n = 5) and  $55.4 \pm 3.3$  (n = 5), respectively. Further optimization of SPE parameters is being carried out to improve % recovery of nAg from aqueous sample. Centrifugal filtration with 3 KDa molecular weight cut-off cellulose based filter isolates nAg from its dAg fraction effectively. Developed analytical methodologies employing CNT based SPE, centrifugal filtration and ICP-MS will be utilized to investigate the fate of nAg in aquatic mesocosms as part of the Lake Ecosystem Nanosilver (LENS) project in the Experimental Lakes Area (ELA) of Ontario, Canada.

**WP261 chemistry and transport of silver nanoparticles in fresh water applications** L. Ellis, University of Birmingham. Laura-Jayne Ellis, Mohamed Baalousha, Jamie R. Lead. University of Birmingham, School of Geography, Earth and Environmental Sciences. Nanoparticles (NPs), defined as solid materials within the range of 1-100nm in size, have unique physicochemical characteristics (Fiorino, 2010). One of the most popular manufactured NPs is silver (Ag) due to its anti-bacterial properties, and low production cost, (Fabrega *et al*, 2011). A number of studies (e.g. Benn and Westerhoff (2008)) have identified that these NPs are released from consumer products. The increased use of AgNPs in such products will result in increased concentrations of dissolved silver in the environment. Therefore

it is important to understand the transformations of these particles released into fresh water systems and their environmental fate. We have synthesised and characterised a range of AgNPs of different sizes (e.g. 7-40 nm) and surface coatings (e.g. citrate, PVP and PEG). Selected particles were exposed to different environmentally relevant conditions such as UV light, fulvic acid and ionic strengths over periods of days to weeks. The temporal changes in properties and partitioning are discussed.

**WP262 Determination of annual loads of inorganic atmospheric contaminants in northern Alaska by measurement in snowpack and passive ion collectors** B.G. Brumbaugh, US Geological Survey / Columbia Environmental Research Center, US Geological Survey; S. Earhart, Five Rivers Services, LLC; G. Linder, USGS/BRD/Columbia Environmental Research Center, HeronWorks Field Office, USGS/BRD/CERC / HeronWorks Field Office; P. Neitlich, National Park Service. Protected lands in Alaska managed by the U.S. National Park Service (NPS) are under increasing threats from atmospheric pollutants. At remote locations the use of conventional systems for monitoring deposition of atmospheric pollutants over extended time intervals is generally not practical; consequently, sampling methods that require minimal maintenance and no power for operation are needed for use in monitoring these areas. Passive sampling for selected ionic pollutants has been reported experimentally using specially adapted, ion-exchange collectors (IECs) in limited studies of forested ecosystems; this approach shows promise for monitoring loads of ionic contaminants from both wet and dry deposition (Fenn and Poth 2004; Simkin *et al.* 2004). However, this methodology has been attempted at relatively few sites and it has not been demonstrated in arctic environments. Furthermore, the very low concentrations of the ionic contaminants that are typically present in precipitation of pristine regions of northern Alaska are expected to present increased challenges for this methodology. We determined annual loads of ammonium, nitrate, sulfate, and selected trace metals at three sites in northern Alaska, based on two sample collections for each site. Winter snowpack was sampled just before onset of thaw in late March; while IECs were used for passive, integrative sampling from wet and dry precipitation between March and October. One of our sites was located within the city of Fairbanks; the other two were co-located with National Atmospheric Deposition Program stations – one in Denali National Park, the other in the Caribou-Poker Creeks Research Watershed study area located 45 km north of Fairbanks. Two-stage IECs were developed for this study, which produced much lower ammonium ion concentrations in field blanks as compared to a single-stage design reported previously in the literature. Triplicate snow samples and triplicate IECs were used for measurement at each site. Also deployed at each site were three IEC field blanks and one field-spiked IEC. Accuracy – by comparison to co-located NADP data; precision, detection limits, and practical considerations are discussed for this approach.

**WP263 Documenting Natural Background Concentrations of Nutrients, Trace Metals and Bacteria in Reference Streams in San Diego, Orange Counties, California** L. Tiefenthaler, Southern California Coastal Water Research Project / Watersheds, SCCWRP / Biogeochemistry; M. Sutula, SCCWRP / Biogeochemistry; J. Griffith, SCCWRP / Microbiology; J. Shrake, AMEC, Environment & Infrastructure; R. Siramanne, AMEC, Environment & Infrastructure / CPSWQ Water Resources; M. Raith, SCCWRP / Microbiology; J. Burns, AMEC, Environment & Infrastructure / CPSWQ Water Resources; C. Pham, SCCWRP / Biogeochemistry. The streams of Orange and San Diego County watersheds are an important natural resource for the region, providing critical natural habitat and serving as refuge, foraging areas and breeding groups for a number of threatened and endangered species. Changes in watershed runoff over the past century have resulted in increased wet and dry weather runoff to streams, which bring increased loads of bacteria, nutrients, metals and other contaminants. As a result, many streams in the region have been placed on the State's 303(d) for impairment of beneficial uses, requiring adoption of total maximum daily loads (TMDLs). An important component of a TMDL is the numeric target, which established the limit of the contaminant required to achieve beneficial uses. Progress on TMDLs in the San Diego Regional Water Quality Control Board's (SDRWQCB) region is currently hampered by the lack of a consistent set of scientifically-defensible numeric targets for streams. Existing standards do not account for natural sources of constituents. One approach to developing numeric targets that account for "natural sources" is to establish the concentrations or loads from streams in a minimally disturbed or "reference" condition. To address this data gap, the Counties of



San Diego and Orange, their municipal stormwater co-permittees, and the City of San Diego have collaborated on the support of a regional stream reference study. The goal of this project is to collect the data necessary to derive reasonable and accurate numeric targets for bacteria, nutrients, and metals, based on a reference approach and to provide a scientific basis for selecting numeric targets during a TMDL. The general approach to be used in this project involves the measurement of wet and dry weather flows and constituents at "reference" sites. Six wet weather sites will be monitored three times, for a total of 18 site events, while 10 dry weather sites will be monitored on a weekly to biweekly basis over a year. The study seeks to answer three key questions: 1) How does the exceedence frequency vary between summer dry weather, winter dry weather and wet weather?, 2) How does the exceedence frequency vary by hydrologic factors, including: size of storm (wet weather only), 2) discharge flow rate and volume (wet and dry weather), and 3) How does the exceedence frequency vary by early versus late season storm. This poster will present the preliminary results of Year 1 wet and dry weather sampling for this project.

**WP264 Effects of humic substances extracted from river sediments and soils on the algal growth inhibition induced by copper ecotoxicity** H. Kurokawa, Tokyo National College of Technology; R. Shoji, Tokyo National College of Technology. In general, the ecotoxicity induced by copper depends on pH, the concentration of ions and humic substances. Humic substances are the end product of decaying organic matter in nature. Humic substances affect on the mobility and bioavailability of cupric ion in aquatic and soil system. In addition, characterization of humic substances varies according to its origin. In order to estimate the ecotoxicity induced by the copper in aquatic and soil, it is important to analyze cupric ion binding by humic substances. In this study, humic acids extracted from river sediments or clay soil are as a source of humic substances. Bindings of proton and cupric ion to the humic acid are analyzed by the NICA (Non-Ideal Competitive Adsorption)-Donnan Model. The objective of this study are to analyze the binding of proton and cupric ion to three types of binding sites in the humic acids, and to examine the influences of each humic acids on the copper growth inhibition to *Pseudokrichneriella subcapitata*. The amount of phenolic sites in humic acids was larger than that of carboxylic sites in terms of the binding sites for proton and cupric ion to each humic acid. In terms of binding cupric ion to the humic acid extracted from river sediments were different than the humic acid extracted from clay soil. It was difficult to figure out copper binding in the humic acids extracted from clay soil by the NICA -Donnan Model. The most controlling factor for the prediction seems to be the molecular weight and the volume of the donnan phase of humic acids. In terms of the influences of the humic acid on copper growth inhibition to *P. subcapitata*, the humic acid extracted from river sediments were not significantly different than the humic acid extracted from clay soil.

**WP265 Evaluating the impact of smelting activities on soils and *Pinus echinata* within the downwind air shed of a Zinc Smelting Plant, Palmerton, PA** J.J. New, E. Myers, Towson University / Urban Environmental Biogeochemistry Laboratory; S.M. Lev, Towson University / Urban Environmental Biogeochemistry Laboratory, Towson University / Professor. The New Jersey Zinc Company operated a Zinc Smelter from 1898 until 1980 in Palmerton, Pennsylvania. At the peak of operations, an estimated 47 tons of Cadmium (Cd), 95 tons of Lead (Pb), and 3,575 tons of Zinc (Zn) were released to the atmosphere annually. Effected areas immediately adjacent to the site have been remediated however the impact of the Palmerton smelter on the downwind air shed has not been carefully investigated. This study examined the impact of the Palmerton Smelter on the air shed along a 40 km downwind transect. Soil cores and tree cores from *Pinus echinata* were collected at 6 locations along a downwind transect to examine the distribution of smelter derived metals in soils and the impact of those metals on *Pinus echinata*. *Pinus echinata* was selected as the study species because conifers have proven useful as biomonitors in previous dendrochemical studies and are ubiquitous in the Palmerton air shed. Soils were analyzed for bulk composition and subjected to sequential extractions to evaluate the concentration and form of trace metal constituents. Tree cores were analyzed for growth rate and chemical composition using Laser Ablation ICP-TOF-MS. Results from the analysis of soils from within the Palmerton air shed indicate that Zn concentrations increase downwind of the Palmerton site, in some instances exceeding the TEC for land plants (101 mg/kg). Trends in tree ring growth data from downwind sites with in the Palmerton air shed exhibit increased growth rates after the cessation of smelting activities in 1980.

Zn uptake and storage by trees in the air shed are poorly correlated with Zn soil concentrations suggesting that the impact on growth rates may not be related to soil chemistry and may more likely be correlated with uptake from dry deposition of atmospheric particulates during active smelting operations.

**WP266 Metal Contamination in the Cordillera Blanco Mountain Range of Central Peru** R. Combs; R.M. Sofield, Western Washington University / Huxley College of Environment. Some of the world's largest metal mining operations are located at high elevations in the Andes of Peru. This provides a unique situation where non-volatile metals may be transported into high alpine mountain systems from the open pit mines. As part of the 2012 Climber Scientist program, we will collect snow and water samples from the Ishinca and Llaca valleys of the Cordillera Blanco Mountains. Dissolved and total recoverable metals will be analyzed along with dissolved organic carbon concentrations. Field measurements of lakes and streams will include alkalinity, pH, and hardness. These results will be compared to US EPA Water Quality Guidelines as a way to guide understanding of the impacts of the mining activities. This expedition is expected to produce a baseline of data that can be used by Peruvian scientists, planners, and managers to make informed decisions about their natural resources. The results will be presented at the 33<sup>rd</sup> Annual SETAC North America meeting.

**WP267 Nickel Beyond Environmental Exposure: Stainless Steel Cookware's Contribution to Nickel Exposure from Cooked Foods** K.L. Kamerud, Oregon State University; K.A. Hobbie, Oregon State University / Environmental Molecular and Toxicology, Oregon State University / Department of Environmental & Molecular Toxicology, Oregon State University; K.A. Anderson, Oregon State University / Environmental & Molecular Toxicology, Oregon State University / Dept. of Environmental & Molecular Toxicology. Environmental and occupational exposure to nickel is generally well characterized, however other potential routes of exposure, such as leaching from stainless steel cookware are not well known. Nickel occurs naturally in plants and animals, and humans are exposed to it from these dietary sources. Humans are estimated to ingest an average of 150 to 900µg of nickel per day. In 2001, the Tolerable Upper Intake Level (UL) of nickel was decreased to 1000µg per day. The toxicological studies of nickel indicate that single oral doses as low as 3000µg causes allergic dermatitis. It is recommended that individuals sensitive to nickel lower their exposure. However, nickel may be introduced to the diet via leaching from stainless steel cookware into foods during cooking processes, contributing to flare-ups of allergic contact dermatitis. We tested three variables, grade of stainless steel, cook time, and repeated usage or cooking cycles, for their effects on nickel leaching from stainless steel during the process of cooking tomato sauce. Sampling involved simulated home cooking procedures with stainless steel grades equivalent to those found in cookware. After the cooking procedure samples underwent acid digestion, and analysis using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). Results show significant differences in nickel leaching between grades of stainless steel, indicative of the reported protective properties of the chromium oxide film in stainless steel. The amount of nickel in tomato sauce increased from 4.99mg/kg after a cook time of two hours to 7.63mg/kg after a cook time of twenty hours. The first cooking cycle showed the highest nickel concentration of 5.76mg/kg. Sequential cooking cycles with the sample stainless steel resulted in decreasing amounts nickel leached with each subsequent cooking cycle. From this set of experiments we can conclude that in addition to dietary exposures, stainless steel comparable to cookware is a source of nickel. Nickel exposure from stainless steel is variable, and dependent on grade of steel, cook time, and number of cooking cycles.

**WP268 Optimizing recovery of selenium from a range of biological matrices using a nitric acid digest and detection by HGICP-MS** A. Kleckner, U.S. Geological Survey; A.R. Stewart, U.S. Geological Survey / Water Resources Division; E. Kakouros, US Geological Survey. Selenium (Se) concentrations in fish tissues that are being considered for regulatory criteria span a very narrow range (4.7-7.3 µg/g dry wt.). This narrow range assumes that analytical methods for the measurement of Se are both accurate and precise. Existing wet digestion methods involve the chemical degradation of sample matrices in solution, usually with a combination of acids to increase solubility. The complete dissolution and decomposition of organic matter is required for the release of all Se. Digestion with nitric acid and perchloric acid (HNO<sub>3</sub> + HClO<sub>4</sub>) is a proven, highly effective, and precise method for digesting selenium. However, the use of HClO<sub>4</sub>



requires extreme caution and special venting facilities that are not widely available. Here we evaluate a multi-step wet digestion, using a combination of  $\text{HNO}_3$ ,  $\text{HCl}$ ,  $\text{BrCl}$ , and  $\text{H}_2\text{O}_2$ , as an alternative to  $\text{HClO}_4$ . The challenge of using  $\text{HNO}_3$ , which has lower oxidative power compared with  $\text{HClO}_4$ , is achieving complete digestion of organic complexes. To overcome this, samples were heated in concentrated  $\text{HNO}_3$  in closed Teflon vials that provided minimal headspace, and were followed by treatment with other oxidizers. We evaluated the effectiveness of our method to digest different tissue matrices by using a variety of certified reference materials (CRM): DORM-2, DOLT-3, NIST 2976, TORT-2, and PACS-2. Our method requires very small sample masses (10-50 mg) and digestates are mixed with concentrated  $\text{HCl}$  to reduce the selenium to  $\text{Se}^{\text{IV}}$ , the most favorable valence for hydride generation. Due to the difficulty of finding an internal standard that mimics both the hydride generation reaction and the various matrix suppression effects, a standard Se solution of 5 ppb was measured every 5 to 6 samples to correct for signal drift. The signal drift was corrected based on the sample run time and the corresponding value for the 5 ppb standard using a polynomial equation and the signal value for the same standard from the calibration curve calculated using the calibration coefficient. The measured concentrations of Se in CRMs agreed well with certified values after the drift correction. The method detection limit (MDL) was 0.21 ppb and 0.08  $\mu\text{g/g}$ . This method corrects for the low bias (8-10%) observed in the literature using a  $\text{HNO}_3 + \text{H}_2\text{O}_2$  digestion and allows for consistent recoveries achievable in most laboratory settings.

**WP269 Photocatalytic activity of magnetically recyclable  $\text{Fe}_3\text{O}_4/\text{TiO}_2$  in the color removal of a triphenyl methane dye: A preliminary investigation** U. Nascimento, Institute of Chemistry of Sao Carlos (USP) / Chemistry and Molecular Physics Department; L. Varanda, E. Bessa, Institute of Chemistry of São Carlos; R.N. Padovan, University of Sao Paulo / Instituto de Química de Sao Carlos / Departamento de Química e Física Molecular. The application of semiconductors for treating polluted waters and wastewaters is a promising environmental remediation technology, especially for organic pollutants. Among several oxide semiconductor photocatalysts,  $\text{TiO}_2$  is the most suitable material for a widespread environmental application, due to its biological and chemical inertness, strong oxidizing power, cost effectiveness, and stability regarding photo and chemical corrosion. However,  $\text{TiO}_2$  also has some disadvantages, such as: it is UV-excited and it is usually necessary an additional step (e.g. filtration or centrifugation) for recycling purposes. In trying to solve those drawbacks, we fabricated a magnetic photocatalyst  $\text{Fe}_3\text{O}_4/\text{TiO}_2$  core/shell submicroparticles with high surface area and good photocatalytic activity. We used Acid Blue 9 (C.I. 42090) dye color removal for investigating the photocatalytic activity, pH and catalyst dosage effects through a  $2^2$  factorial design. Statistical analyses were performed in order to assess the significance of the parameters used. We obtained magnetically recyclable  $\text{Fe}_3\text{O}_4/\text{TiO}_2$  core/shell submicroparticles with specific surface area of 80  $\text{m}^2/\text{g}$ . The magnetic photocatalyst presented a red shift in the absorbance spectrum. Both parameters (pH and catalyst dosage) were statistically significant. It is possible to reach a complete color removal of the dye solution with smaller pH values and higher catalyst dosages. In these preliminary study, the greatest color removal (83%) was achieved with pH 3.0, 1.0  $\text{g L}^{-1}$  of photocatalyst, and 1 h of reaction. The photocatalyst could be easily removed from suspension by a simple magnet.

**WP270 The Determination of Heavy metals in Soils, Plants and Hand-dug Wells from an Electronic Waste Disposal site in Lagos, Nigeria** A.O Bankole, J.M. Godoy, Pontificia Universidade Católica do Rio de Janeiro, Brasil / Department of Chemistry; D.V. Perez, Embrapa Soils, Rio de Janeiro, RJ, Brazil; O. Osibanjo, University of Ibadan, Ibadan, Oyo State, Nigeria. / Department of Chemistry. The disposal of e-waste is a global environmental issue, as these wastes contain a wide array of toxic substances among which are heavy metals. This study examined heavy metal contamination of soil, hand dug wells, plant roots, shoots, leaves and flowers from an e-waste disposal area in Alaba International Market, Lagos, Nigeria. Surface and core soil samples were collected, wet digested using the EPA 3051A method. The ICP-OES and FAAS spectrophotometric techniques were used for analysis of heavy metals. Soils were physically characterized for pH, organic matter and mechanical properties. The experimental study was conducted using the BCR 141r standard reference material characterised for aqua regia available recoverable trace elements and NIST 1547 peach leaves which all gave good recoveries for all element studied. The levels

of Pb, Zn, Ni, Cu, Cd and Cr in  $\text{mg/kg}$  ranged from 16.670-6520.683, 61.290-3451.089, 2.944-716.391, 14.289-11966.698, 1.167-138.250 and 4.738- 116.680 respectively. The study also recorded high concentrations of heavy metals in plants and groundwater above the maximum allowable level by WHO/FAO. Results from the study established heavy metal soil contamination, heavy metal groundwater contamination and heavy metal bioaccumulation in plants which far exceeds other concentrations recorded in previous studies in other market region. A more detailed study is underway to study the metal speciation in order to understand the environmental behaviour of these potentially toxic elements. It is concluded that indiscriminate dumping and burning of e-waste is a principal source of heavy metal contamination.

**WP271 Tributyltin and Its Derivatives in Water and Sediment Samples of National Inland Waterway Authority Harbour of Warri, Nigeria** E.T. Ogbomida, National Centre for Energy and Environment / Ecotoxicology and Environmental Forensics; L.I. Ezemonye, University of Benin / Zoology Dept; A.A. Enuneku, I. Tongo, University of Benin / Animal and Environmental Biology. Tributyltin (TBT) is an organotin toxic compound commonly used as an antifouling agent in marine paint biocide formulation. Due to its wide industrial application and its consequent discharge into the environment, TBT pollution has been recognized as a major environmental problem at global scale. Recently, TBT has been considered to be the most toxic substance ever deliberately introduced into the environment and has been known to be extremely poisonous to mollusc fishery resources. Contamination of tributyltin (TBT) and its derivatives dibutyltin (DBT) and monobutyltin (MBT) in surface water and sediment samples of National Inland Waterway Authority (NIWA) Harbour in Warri, Nigeria was assessed by quantitative determination of tributyltin and its derivatives using gas chromatography coupled with flame ionization detector (GC-FID) with detection limit of 0.001  $\mu\text{g/l}$  after derivatization with Grignard reagent pentyl magnesium chloride. The concentrations of TBT in surface water samples ranged from 0.01  $\mu\text{g/l}$  to 0.05  $\mu\text{g/l}$ , while its derivative DBT and MBT ranged from 0.01  $\mu\text{g/l}$  to 0.03  $\mu\text{g/l}$  and 0.01  $\mu\text{g/l}$  to 0.02  $\mu\text{g/l}$ . In sediment samples TBT ranged from 0.03  $\mu\text{g/g}$  to 0.05  $\mu\text{g/g}$  while its derivatives DBT and MBT ranged from 0.01  $\mu\text{g/g}$  to 0.03  $\mu\text{g/g}$  and 0.01  $\mu\text{g/g}$  to 0.04  $\mu\text{g/g}$ . The total butyltin concentration ranged from 0.04  $\mu\text{g/l}$  to 0.09  $\mu\text{g/l}$ . TBT was generally dominant in all the samples, suggesting fresh inputs of tributyltin compounds and/or less degradation of TBT. The Butyltin degradation index (BDI) for the National Inland Waterway Authority (NIWA) Harbour in water ranged from 0.6 to 6 and in sediment 0 – 1.5 indicating a lot of fresh input of butyltins into the harbour and a lower degradation rate. The observed mean concentrations TBT in surface water and sediment samples exceeded the ecotoxicological benchmark of 0.01  $\mu\text{g/l}$  recommended by USEPA and are severe enough to cause harmful effects on the harbour flora and fauna. This survey provides first information and documentation of tributyltin compound and its derivatives contamination in National Inland Waterway Authority (NIWA) Harbour and suggests further environmental monitoring of other Nigeria harbours. Therefore, there is a need to regulate the use of butyltins as biocides in marine antifouling paints.

**WP272 Whole-sediment AVS-SEM Analytical Reference Standard – A Synthetic Alternative** A.M Gonzalez, Envonyx Environmental Technologies / Environmental Services, Independent Research & Development / Environmental Services. Acid-volatile sulfide (AVS) and simultaneously extractable metals (SEM) have proven to be robust concepts for explaining and predicting the responses of aquatic organisms after exposure to sediment contaminated by certain transition metals. In the presence of excess reduced sulfur species, the cumulative chemical activity (and by association, the toxicity) of transition metals in pore water is suppressed by metal-sulfide equilibria. Accurately judging a metal-contaminated sediment sample as hazardous or innocuous to aquatic organisms depends in large part on analytical precision and bias. The quality of environmental data is validated using quality assurance procedures (e.g., running reference standards) in parallel with the primary analyses. One factor potentially affecting the accuracy of some analysis (e.g., AVS and SEM) is matrix interference effects. The ideal reference standard for AVS-SEM analysis would be a temporally stable material with precisely known concentrations (of AVS and associated metal[s]) that responds consistently and predictably to analytical procedures, and that replicates the matrix background of natural sediment samples. Regrettably, the species that comprise AVS in natural sediment are only stable under a narrow set of conditions, and can be altered by changes in

environmental conditions (e.g., oxidation). Natural sediment containing AVS and SEM is too unstable to serve as a reliable reference standard for AVS and SEM measurements. To address this deficiency, the author investigated the possibility of creating an analytical reference standard entirely from off-the-shelf materials, based on current work with synthetic sediment substrates and a literature review of AVS-SEM chemistry. A number of chemicals and minerals were screened as potential components of a synthetic reference standard. Various combinations of a short-list of potential materials were evaluated against the ideal reference standard criteria mentioned previously. Three metal sulfides (CaS, ZnS and Na<sub>2</sub>S) and two synthetic sediment compositions were analyzed for AVS and SEM over a three month period. Results show that ZnS in either synthetic sediment matrix gave nearly quantitative recovery of both sulfide and zinc over the entire study period. Zinc sulfide in a synthetic sediment matrix may be a viable reference standard for AVS and SEM analyses.

**WP273 Selenium cycling in an estuarine ecosystem receiving inputs from coal-fired power stations** L. Schneider, University of Canberra; W.A. Maher, University of Canberra / Dept. of Health, Design & Science; E. Krikowa, University of Canberra; J. Potts, NSW Environment and Heritage; B. Gruber, University of Canberra; A. Chariton, CSIRO Land and Water. Release of trace elements, especially selenium, from coal power plant fly ash is of concern due to the potential for environmental contamination and accumulation in food webs. Lake Macquarie has two power stations located on its shore and has been contaminated with selenium (Se), cadmium (Cd), lead (Pb) and zinc (Zn). The presence of these elements are a direct result of runoff from nearby coal power plant fly ash. This project investigated the history of trace metal contamination in sediments in Lake Macquarie using Pb<sup>210</sup> analysis to evaluate whether selenium concentrations have decreased after improved fly-ash handling procedures have been implemented at the power stations. Selenium accumulation and biomagnification in the seagrass food webs were examined by using C and N isotopes to establish food chains. As well the volatilization of selenium from sediments was measured. This work clarifies the current and historical inputs of selenium and characterizes how selenium may be lost from sediments by volatilization or remobilization into marine food webs.

**WP274 The NOAA perspective: addressing marine plastics through science and policy implementation** C.D. Arthur, National Oceanic & Atmospheric Administration / Marine Debris Program, National Oceanic and Atmospheric Administration / National Ocean Service; S.M. Lippiatt, P. Murphy, N.E. Wallace, National Oceanic and Atmospheric Administration / Marine Debris Program. Anthropogenic items enter the marine environment from a variety of sources, often as a result poor waste management systems, and attract attention due to high visibility when floating in surface waters. Plastic materials maintain capability for sorption, long-range transport, and ingestion by an increasing number of aquatic organisms. As a result, plastic debris may be considered an emerging contaminant in aquatic systems. Chronic effects are likely to be compounded by the longevity of plastic materials in marine systems. Thus, continued research into this global phenomenon is necessary to develop appropriate long-term solutions and mitigation strategies. This presentation will detail NOAA Marine Debris Program efforts to address the issue of marine plastics through both science and policy implementation. A focus will be placed on describing (1) a new information clearinghouse on marine debris that will enhance communication and sharing of best management practices among researchers, and (2) applications of current research to policy frameworks such as the Honolulu Strategy on marine debris.

**WP275 Quantification of toxic metals leaching from macro-plastic litter on beaches** E. Nakashima, Ehime University, Ehime University / Centre for Marine Environmental Studies (CMES); A. Isobe, S. Kako, Ehime University / Center for Marine Environmental Science and Studies (CMES); T. Itai, Ehime University / Center for Marine Environmental Studies (CMES); S. Takahashi, Ehime University / Center for Marine Environmental Science and Studies. The potential risk of toxic metals leaching into a beach environment from macro-plastic litter washed ashore (Ookushi beach, the Goto Island, Japan) was examined by balloon aerial photography, *in-situ* beach surveys, and leaching experiments in conjunction with a Fickian diffusion model analysis. Using a X-ray fluorescence analyzer (XRF), five toxic metals, chromium (Cr), cadmium (Cd), tin (Sn), antimony (Sb) and lead (Pb) were detected in macro-plastic litter collected during the beach surveys.

In particular, fishing floats made from polyvinyl chloride (PVC) showed the greatest quantity of Pb. The balloon aerial photography in conjunction with the beach survey demonstrated that the mass of Pb derived from plastic litter was 313±247 g. Furthermore, Pb leaching experiments of collected PVC floats concluded that Pb in the macro-plastic litter leached into surrounding water on the actual beach, and that macro-plastic litter acted as a "transport vector" of toxic metals to the beach environment. Using the experimental data, the total mass of Pb leaching from the macro-plastic litter over a year onto Ookushi beach was estimated. The detail results received up to this date will be presented; our result suggests that toxic metals derived from macro-plastic litter are a potential threat to the beach environment due to their accumulation in beach soil over time.

**WP278 Calculating the Swedish economy-wide emissions of substances from plastic materials and fibers** S. Molander, Chalmers University of Technology / Environmental Systems Analysis, Chalmers University of Technology; K. Fransson, Chalmers University of Technology / Environmental Systems Analysis; P. Haglund, T. Holmgren, University of Umea / Chemistry; T. Rydberg, IVL Swedish Environmental Research Institute; J. Tivander, Chalmers University of Technology / Environmental Systems Analysis; J. Westerdahl, IVL Swedish Environmental Research Institute. "Plastics" are very diverse, multipurpose and ubiquitous materials found in very many types of products like shoes, cars, bags and containers. Plastic materials have these many uses due to the possibility of modifying the polymer matrices constituting the bulk material in very many ways with a large number of additives; compounds that are more or less permanently attached in the matrix. Some of these additives have properties with high importance for the final functionality of the product. Flame-retardants are a well known example of an additive making otherwise combustible plastic materials (often textile fibers) much less apt for taking fire. There are however a very large number of substances which have got less attention. Our modeling approach aims at a quantification of emissions from a large set of materials occurring in a typical developed country – Sweden. The approach is not based on a back-calculation of measured emitted substances on an inventory of substances in products. Instead it is "bottom-up" in the sense that it is a combination of a diffusive mass-transfer emission model with models providing information on aggregated product surface areas and material composition of these areas. The physico-chemical modeling of substance release from a surface under a specific set of environmental conditions relies on substance and material characteristics, while the substance and material flow models are based on trade statistics, longevity data and product properties. The approach is a unique emission model giving the possibility to feed in results to environmental fate modeling and environmental analysis. Results show that textiles, many constituting plastic polymer fibers like polyester or polyamide, and the huge number of organic substances occurring in them, are of particular interest due to the fact that this product category constitutes the largest surface area. Due to wear and washing textiles are also fragmented, giving very small fiber fragments contributing to a further increased surface area that increase the emission rate from the fiber materials in textiles. The results indicate that a number of substances, beside known environmental pollutants, are emitted from textiles.

**WP279 Toxic Chemical and Plastic Fragment Pollution the Pacific Ocean** J. Greene, California State University, Chico / Mechanical Engineering and Sustainable Manufacturing. Plastics comprised the majority of collected waste in worldwide beach clean-ups in 2006, 2007, 2008, 2009, and 2010. The most common sources of beach litter were related to smoking and recreational activities around beaches in the United States, Canada and Mexico, accounting for over 80% of the debris. In California, Washington, Oregon, and Hawaii the five most common plastic debris items on beaches are cigarette filters, food wrappers and containers, beverage caps and lids, bags, and food service items, e.g., cups, plates, and cutlery. The majority of plastic items are made from four common plastics; polyethylene, polypropylene, polystyrene, and PET, accounting for 75% of the plastic debris. Pre-production plastic pellets also account for significant amounts of plastic in the oceans from storm run-off of industrial areas. The fate of plastics in the oceans can lead to fragmentation and result in slurry of plastic particles that can degrade and release toxic chemicals in to the oceans. The most persistent chemical released from plastics is Phthalates which can come from vinyl (PVC) plastics and from recycled plastic bottles of PET, PP and HDPE. Phthalates are found in lotions, creams, shampoo, and soaps that are placed in plastic bottles. Other toxic chemicals include, flame

retardants, BPA, antimony oxide, heavy metal inks, and styrene monomer as the plastics break down. Plastics can accumulate toxins floating in the oceans from persistent organic pollutants (POPs). POPs can include DDT, hexachlorobenzene, polychlorinated biphenyls (PCB), polycyclic aromatic hydrocarbons, among others. Biodegradable plastics can offer a safe substitute since they do not have the traditional additives or components that are toxic. PHA biodegradable plastics are the only biodegradable plastic that demonstrates biodegradation in ocean water.

### **WP280 Plastic Marine Pollution in the South Pacific Subtropical Gyre**

M. Eriksen, 5 Gyres Institute. Plastic marine pollution in the open ocean of the southern hemisphere is largely undocumented. Here, we report the result of a (4489km) 2424 nautical mile transect through the South Pacific Subtropical Gyre. Neuston samples were collected at 48 sites, averaging 50 nautical miles apart, using a manta trawl lined with a 333µm mesh. The transect bisected a predicted accumulation zone associated with the convergence of surface currents, driven by local winds. The results show an increase in surface abundance of plastic pollution as we neared the center and a decrease as we moved away, verifying the presence of a garbage patch. The average abundance and mass was 26,898 particles/km<sup>2</sup> and 70.96 g/km<sup>2</sup>, respectively. 88.8% of the plastic pollution was found in the middle third of the samples with the highest value of 396,342 particles/km<sup>2</sup> occurring near the center of the predicted accumulation zone.

### **WP281 Understanding and Minimizing the Health Risks of Plastics**

R.U Halden, Arizona State University / Center for Environmental Security, The Biodesign Institute at Arizona State University. Today, the worldwide annual production of plastics surpasses 300 million tons. Modern society heavily relies on products made from plastics, including various lifesaving applications in human medicine. Yet, plastics also pose various environmental and human health risks. A key concern centers around endocrine disrupting properties of plastics elicited by compounds including bisphenol A (BPA) and di-(2-ethylhexyl) phthalate (DEHP). Despite half a century of research on various ingredients of plastics, the scientific community remains divided concerning the real and perceived safety risks these versatile compounds pose. This presentation summarizes information from over 130 peer-reviewed publications on health effects of plastics in animals and humans. It identifies potentially problematic exposures of susceptible populations and also examines adverse environmental impacts caused by plastic pollution. Finally, it introduces the concept of the “5 Rs” (*reduce, reuse, recycle, rethink, restrain*) that may assist humanity in moving toward resource conservation and a more judicious selection of plastics for use in daily life with the goal of minimizing unwanted and potentially harmful exposures of humans and wildlife alike.



**RP001 Identification, Quantification and Evaluation of Emerging Contaminants of Concern in the Great Lakes Fish Monitoring and Surveillance Program** B. Crimmins, Clarkson University; M. Milligan, SUNY Fredonia; J. Pagano, SUNY Oswego; P. Hopke, T.M. Holsen, Clarkson University. The Great Lakes Fish Monitoring and Surveillance Program has a long history of evaluating the presence of anthropogenic chemicals in Great Lakes fish. Recently, a portion of the program has been devoted to the identification, evaluation and quantification of emerging contaminants in the Great Lakes system. Utilizing complementary screening techniques, the current program is in the process of; 1) developing a chemical contamination database using GCxGC-ToF mass spectrometry for Great Lakes trout; 2) identifying aerobic and anaerobic breakdown products of emerging contaminants to identify true chemicals of concern; 3) developing validated methods to quantify newly found emerging contaminants; 4) developing hybrid visualization techniques utilizing APGC and UPLC-QToF MS<sup>c</sup> technologies. Challenges and successes will be presented for each approach with an evaluation of current needs and exploration direction.

**RP002 Utilization of GCxGC-TOF Mass Spectrometry for the Identification of Persistent and Bioaccumulative Emerging Contaminants in the Great Lakes Region** M. Milligan, SUNY Fredonia / Department of Chemistry; D. Richards, A. Conrad, SUNY Fredonia; B. Crimmins, X. Xiayan, T. Holsen, P. Hopke, Clarkson University; J. Pagano, SUNY Oswego. We have been employing GCxGC with time-of-flight (TOF) mass spectrometry to screen for persistent and bioaccumulative emerging contaminants in the Great Lakes region using top predator fish as bio-monitors. Whole fish composites of Great Lakes Lake Trout for all of the five lakes were extracted using accelerated solvent extraction. These extracts were then subjected to a series of fractionation steps using gel permeation chromatography, deactivated silica, acidic silica, and carbon columns to isolate potential candidates, followed by analysis using GCxGC-TOF mass spectrometry. Mass spectra generated by a deconvolution algorithm were compared to NIST mass spectral libraries for potential hits, which were then assessed on a peak-by-peak basis. Numerous chlorinated, brominated, and non-halogenated potential emerging contaminants have been identified using GCxGC-TOF, such as halogenated dibenzophenones and organotin compounds, and ultimately confirmed with standards. In addition, we have identified a number of chlorinated and brominated compounds that may be the result of the degradation of less environmentally persistent parent compounds.

**RP003 Non-Targeted Analysis of San Francisco Bay Harbor Seals for New Contaminants** J.R. Kucklick, National Institute of Standards and Technology (NIST) / Analytical Chemistry Division, National Institute of Standards & Technology / Hollings Marine Laboratory; S. Klosterhaus, San Francisco Estuary Institute; D.J. Greig, The Marine Mammal Center; J. Murray, National Institute of Standards and Technology / Analytical Chemistry Division. Contaminant measurement in biological samples is almost always done in a targeted fashion using a set list of target compounds and methods specifically geared towards measurement or detection of those compounds. Consequently, the number of routinely targeted compounds analyzable by gas chromatography (GC) with persistent organic pollutant (POP)-like characteristics is probably < 200. Recent theoretical studies based on physical property and usage data suggest that the potential number of POP-like compounds in samples measurable by routine GC/MS methods may be several times this number; however samples are rarely screened for new compounds. Recent advances in analytical instrumentation, in particular two dimensional GC with time of flight mass spectrometry (GCxGC TOF/MS), facilitate the identification of environmental contaminants in complex mixtures. The goal of this study was to use GCxGC TOF/MS to screen harbor seal samples from San Francisco Bay, California for pollutants that may originate within the bay watershed. Blubber samples from six harbor seal samples, five from the San Francisco Bay area and one from a reference site, were extracted, cleaned up by size exclusion chromatography and then analyzed by GCxGC TOF/MS. During the data analysis, legacy parent and metabolite POP spectra were identified and then removed from further consideration to focus on identifying mass spectra originating from novel contaminants. Approximately 20 non legacy POP compounds were detected in each sample including current-use pesticides, halo acetate esters, C8 to C12 chloro- and fluoroalkanes, as well as numerous chloro- and fluoro- ringed compounds. Of these compounds, about half had electron ionization spectra that matched a spectrum in the NIST Mass Spectral Library for a specific compound while half did not. Work is

currently underway to verify the presence of the compounds in the samples by acquiring the individual compounds and reanalyzing the samples by GC/MS. If the presence of a compound is confirmed, it will be quantified in the samples. Work is also in progress on identifying compounds that produced spectra that were not in the NIST library and analyzing seal blood and liver for new polar compounds.

**RP004 Evaluation of toxic compounds in water using bioassay combined with LC-QTOF analysis** A. Jia, B. Clarke, S. Merel, X. Li, S. Wu, University of Arizona; S. Snyder, University of Arizona / Chemical and Environmental Engineering, University of Arizona / Chemical & Environmental Engineering. The risk of human exposed to different water sources brings higher requirement on modern water quality including treated wastewater and recycled water. Conventional chemical monitoring have been criticised on the basis that they cannot include the full range of chemical pollutants that could occur in water, and because they do not account for the combined effects of different chemicals or their transformation products. Bioanalytical tools are complementary to chemical analysis, while they can measure mixture effects and contribute to the assessment of the cumulative effects of chemicals that exhibit the same mode of toxic action and thus concentration additive effects. Furthermore, unknown chemicals including transformation products or byproducts during the treatment process can be identified if they have more toxicity. Bioassay screening combined with instrument identification has been the most efficient way to evaluate the water quality related to human health nowadays. In this study, HLB cartridge tandem Coconut Charcoal was used to maximally extract different species of non-target chemicals from wastewater and recycled water samples. The extractions were evaluated by several cell promoter reporter assay including AR (androgen), ER (estrogen), GR (glucocorticoid), p53, and hypoxia, etc., from Switchgear Genomic, as well as AMESII mutagenicity assay using *Salmonella typhimurium* strains TA98 and TAmix (TA7001-TA7006). Raw, chlorinated, UV irradiated, and ozonated tertiary-treated wastewater samples were collected in Southern Arizona. Significant and unexpected glucocorticoid activity was detected in a wastewater effluent. UV treatment appeared to be the most efficacious at GR activity attenuation. Mutagenicity was also detected in raw and ozone-treated water samples. UHPLC fractionation was further conducted and the fractions were screened through bioassay again in order to identify the possible bio-active compounds. Agilent 6540 Quadrupole Time-of-Flight mass spectrometry (QTOF) was finally used for the chemical identification and the ions were first distinguished from a PCA (Principal Component Analysis) software MPP (Mass Profiling Professional).

**RP005 Preserving the Environment and Toxicity Linkage by Combining Passive Sampling Technologies with Environmental Stressors to Identify Unknown Toxicants** N.D. Forsberg, S.G. O'Connell, Oregon State University / Environmental and Molecular Toxicology; K.A. Anderson, Oregon State University / Environmental & Molecular Toxicology, Oregon State University / Dept. of Environmental & Molecular Toxicology. Regulatory agencies are required to mitigate human and ecological exposure to toxic chemicals of concern. In order to meet this demand, chemical drivers of toxicity and accurate exposure pathways must be identified. Though mixtures are complex systems, it has been postulated that the bioavailable fraction of mixtures is linked to toxicity. Applying additional stressors to chemical mixtures will likely transform the composition of the bioavailable fraction and induce differential toxicological responses. In order to more accurately characterize exposure, regulatory agencies need experimental approaches that can determine the effect of natural/remediation processes on the bioavailable fraction of chemical mixtures and mixture toxicity. Passive sampling devices (PSDs) readily sequester the bioavailable fraction of environmentally relevant mixtures, which includes polycyclic aromatic hydrocarbons (PAHs). We hypothesized that perturbations of PSD extracts via UV light exposure could reduce concentrations of PAHs and simultaneously increase concentrations of currently unmonitored oxygenated polycyclic aromatic hydrocarbons (oxy-PAHs). PSD samples collected from the Portland Harbor Superfund Megasite and the waters of the Gulf of Mexico following the Deepwater Horizon oil disaster were exposed to UV light ( $\lambda = 313 \text{ nm}$ ) for 30 minutes at an irradiance roughly 30-40 times greater than a single sunny day in Northern US cities. Along with PSD samples, 1 ppm standard mixtures of 16 EPA priority pollutant PAHs were also exposed. PAHs and 22 oxy-PAHs were quantified in pre- and post-UV exposed samples using a recently expanded and validated GC-MS analytical method. It was found

that the concentration of nearly half of the monitored PAHs were significantly reduced in standard mixtures following UV exposure, while levels of 9,10-anthraquinone and 7,12-benz[*a*]anthracenequinone were increased by roughly an order of magnitude relative to controls. Similar results were observed in environmentally relevant PSD samples. Combining PSD technology with other environmental stressors could provide a powerful approach for identifying emerging chemicals of concern, accurate exposure pathways, and chemical drivers of toxicity.

**RP006 Characterization of toxic components in aircraft deicer fluids using bioassay directed fractionation and high resolution mass spectrometry** L. Ferguson, Duke University / Department of Civil and Environmental Engineering, Duke University / Nicholas School of the Environment, Duke University / Department of Civil & Environmental Engineering, Pratt School of Engineering / Department of Civil & Environmental Engineering; C.A. Huset, MN Dept of Health / Organic Chemistry Unit; K. Hyland, Colorado School of Mines / Environmental Science and Engineering; S. Geis, A.L. Mager, Wisconsin State Laboratory of Hygiene; D. Cancilla, Western Washington University; S.R. Corsi, U.S. Geological Survey / Water Resources Division. Aircraft deicer and anti-icer formulations (ADAFs) are complex mixtures containing freezing point depressants, corrosion inhibitors, surfactants, dyes, thickeners and anti-foaming agents. Large volumes of ADAFs are used in commercial aviation to remove and prevent ice on aircraft. In previous studies, ADAF toxicity was attributed to benzotriazole corrosion inhibitors and alkylphenol ethoxylate surfactants; however, most of the ADAF toxicity remains unaccounted for. We have performed a toxicity identification and evaluation (TIE) study to identify chemical components of the deicers responsible for inducing aquatic toxicity. Our approach incorporated chemical separation methods to separate ADAF mixtures into discrete fractions which were then evaluated on a three-tier toxicity screening scheme. Screening for toxicity was performed on each fraction by Microtox, *Pimephales promelas* and *Ceriodaphnia dubia* assays. Identification of ADAF components in toxic fractions was performed using chromatographic (HPLC) and mass spectrometric (electrospray Q-TOF MS/MS and MALDI-TOF MS) techniques. Toxic components identified were primarily ethoxylated surfactants, including alkylphenol polyethoxylates, lauryl alcohol polyethoxylates and myristic alcohol polyethoxylates.

**RP007 Comprehensive Analysis of Environmental Contaminants in River Water Using HPLC High Performance Time of Flight Mass Spectrometry** J. Patrick, J. Binkley, K. Siek, LECO Corporation. New classes of compounds are being introduced into the ecosystems every day. These compounds are often later determined to have deleterious effects on the ecosystem. The utilization of non-targeted mass spectrometry in the analysis of environmental contaminants offers several advantages including the ability of selective analysis among comprehensive data and the ability to perform retrospective data analyses based on new information. In the form of high performance time of flight mass spectrometry provides additional capabilities including high resolving power ( $R > 45k$ ) and mass accuracy ( $< 1\text{ ppm}$ ), as well as relative isotope information. In the present study water samples from various locations in a select watershed is analyzed using high resolution UHPLC-TOFMS. Analysis of the samples using high performance time of flight MS has shown in excess of 200 analytes identified by formula with mass errors of approximately 1 ppm. The analytes include many common analyte classes including aromatic amines, halogenated aromatics, pesticides, herbicides and products of consumer origin as well as compounds not currently routinely monitored. High accuracy relative isotope abundances in precursor and fragment ions are used to reduce the number of hits based on the formulas and potential elemental compositions. Exceptionally-high resolving power at lower  $m/z$  ( $>35,000$  at  $m/z = 200$ ) in both precursor and fragment ion modes provided the opportunity to confirm identities consistent with EU guidance using high resolution MS. Other tools including halogen filters and targeted precursor-fragment analyses are also applied to enhance information content at data processing of the comprehensive analysis. The utility of the technique is demonstrated as relates to the potential retrospective evaluation of compounds of interest. The advantages of this over targeted approaches are also apparent.

**RP008 Enrichment free LC-HRMS screening method of anthropogenic sewage pollutants in waste water, receiving water, ground water and drinking water samples** J. Beck, S. Westrup, D. Ghosh, C. Yang, Thermo Fisher Scientific. In last decade, there has been growing public concern

of potential contamination of water and environment with anthropogenic compounds and their degradation products and possible negative influence to the nature and public health. As a response to this fact, there is an increased interest in more efficient screening techniques of larger number of possible pollutants compared to that traditionally carried out by triple quadrupole mass spectrometers. The high resolution and accurate mass does not require optimization of compound specific parameters and has the ability to properly separate matrix from compounds of interest. Fast and highly selective methods are necessary to screen, confirm and quantify different classes of contaminants in complex matrix and at low the analyte concentrations. High resolution mass spectrometry – using full scan analysis mode – has been described as the preferred screening tool due to the possibility to look for a large number of compounds and also because it enables retrospective analysis [1]. Furthermore, the use of a resolving power  $\geq 50,000$  FWHM is reported as being as selective as 2 SRM transitions when using triple quadrupole instruments [2]. In this work, the large volume direct injection has been applied to the screening of anthropogenic sewage pollutants in different matrices using a quadrupole-Orbitrap analyser. The experiment consisted on combining full scan mode at a resolving power of 70,000 FWHM with data dependent MS/MS spectra acquired at a resolving power of 17,500 FWHM. The MS/MS spectra were generated by using a high energy collision induced dissociation cell (HCD). This experiment was tested and evaluated in terms of quantification and confirmatory capabilities for the analyses of anthropogenic sewage pollutants. Different sample types were tested, including waste water, receiving water, ground water and drinking water representing different steps within the water cycle of densely populated and intensively agriculturally used areas.

**RP010 The Great Lakes Environmental Contamination Database Project: A comprehensive tool to support removing beneficial use impairments** J. Peers, Stratus Consulting Inc., Senior Scientist / Stratus Consulting; T. Goeks, NOAA / Office of Response and Restoration; C. Chan, Industrial Economics; D. Smorong, Maven Environmental Consulting Services; P. Myre, Exa Data & Mapping Services. To help improve resource management in the Great Lakes, the National Oceanic and Atmospheric Administration's (NOAA's) Office of Response and Restoration is expanding its existing Query Manager (QM) watershed database with data from state and federal agency partners to expedite de-listing of Great Lakes Areas of Concern (AOCs). The database will be used to assess sediment-related AOC Beneficial Use Impairments (BUIs), support remedial and restoration planning and monitoring, and provide scientific evidence to support sediment-related BUI removal. QM is a freely available database that organizes, standardizes, and provides centralized access to data on environmental chemistry and toxicity. QM is available both as a downloadable desktop application and as a web-based application. Users can easily select, explore, compare, and summarize data using pre-programmed queries. Maps of the query results can be created using associated graphical tools, or the data can be readily exported in a wide variety of formats for use with other data analysis software. To ensure data integrity and comparability, NOAA has established a QM database development process that incorporates rigorous and standardized procedures for checking, processing, integrating, and maintaining data. Given that QM has proven to be a reliable and centralized source and delivery system for compiling, analyzing, and displaying Great Lakes environmental contaminant data, it provides users a powerful tool for coordinating and sharing data and supporting their decision-making.

**RP011 Benthic macroinvertebrates community and sediment toxicity in a metropolitan water supply reservoir in Brazil** F. Lage, A. Brandimarte, University of Sao Paulo – USP / Department of Ecology; C.R. Botta, University of Sao Paulo – USP / Center for Water Resources and Applied Ecology (CRHEA); S.C. Da Silva, University of Sao Paulo – USP / Department of Ecology; E.L. Espindola, Universidade de São Paulo – USP / Escola de Engenharia de São Carlos – EESC. The high demand for water supply led Guarapiranga reservoir, built primarily for power generation, became a relevant public water supply to Sao Paulo city (Brazil) since 1928. Moreover, due to high urbanization, nowadays more than half of its basin area is modified, sewage and diffuse pollution are the main pollution sources. The relationship between benthic community and sediment was evaluated in three sites, in each one of four sampling stations (one near the dam and three in the main reservoir tributaries: Embu-Mirim, Embu-Guacu and Parelheiros). In July 2010, six sampling units for benthic fauna analysis and three for sediment bioassays were collected with an Ekman-Birge grab



in each sampling site. Fauna samples were washed onto a 500 µm mesh sieve and preserved in 4% neutralized formalin in the field. In the laboratory, samples were transferred to 70% alcohol and stained (Rose Bengal). Moreover, two sediment samples of each site were collected with an Ambühl & Bühler corer for physical and chemical measures. All sites had 7 taxa, except Embu-Guacu with 9. Oligochaeta was the most abundant group in all sites, except in Parelheiros where Chaoboridae was most abundant. Silty sediment predominated, except in Embu-Guacu where was a sandy site. The average organic matter sediment content was 35%, with higher value in the dam station. Extremely reducing conditions were observed in Embu-Mirim and near the dam, especially in the drinking water catchment area. In the most sites, the relationship ( $\Sigma [\text{SEM}] - [\text{AVS}] / \text{TOC}$ ) showed improbable toxicity but, in another hand, near the dam we found copper and cadmium values above PEL, Total Nitrogen and Total Phosphorus above the limits of CONAMA 344/2004. Presence of Oligochaeta and Ostracoda was associated with the organic matter content and presence of silt. Besides Chaoboridae, other insect larvae were found, including Tanypodinae, *Chironomus decorus* and Ceratopogonidae. The test with *Daphnia similis* showed significant sediment acute toxicity in all regions except in Embu Guacu. The test with *Chironomus xanthus* and the test for porewater with *Daphnia similis* showed significant sediment acute toxicity in all regions except for one site in Embu Guacu and one in Embu-Mirim. Spatial heterogeneity, due to many of the variables analyzed, divided Guarapiranga reservoir in two compartments: Embu Guacu and Parelheiros; Guarapiranga-dam and Embu-Mirim.

**RP012 Field metal mixture toxicity: linking freshwater biota species diversity to chemical speciation and bioavailability** A. Stockdale, Centre for Ecology and Hydrology / Lancaster Environment Centre, University of Manchester / School of Chemistry; E. Tipping, Centre for Ecology & Hydrology; S. Lofts, NERC Centre for Ecology & Hydrology / Shore Section. Understanding metal and proton toxicity under field conditions requires consideration of the complex nature of chemicals in mixtures. We have previously demonstrated a novel method that relates streamwater concentrations of cationic metallic species and protons to a field ecological index of biodiversity (EPT species richness). The model (WHAM- $F_{\text{TOX}}$ ) has also been used to assess regional temporal trends involving comparison of modeling and observations. This presentation communicates a new application of this model to lake zooplankton species richness. The field indicator used in this work was the combined richness of Cladocera and Copepoda zooplankton species. The new modelling has been undertaken with over 1000 data from surveys of 185 individual lakes in 7 different geographic regions. We present data for general model fitting and details from individual regions, including temporal trends.

**RP013 In situ assessment of time-integrated dissolved concentrations and toxicity of metals during diel cycling in streams** L. Balistrieri, D. Nimick, U.S. Geological Survey; C. Mebane, US Geological Survey / NOAA Fisheries Liaison, US Geological Survey/WRD / NOAA Fisheries Liaison. Evaluating water quality and the health of aquatic organisms is challenging in systems with systematic diel (24 hour) or less predictable runoff-induced changes in water composition. To advance our understanding of how to evaluate environmental health in these dynamic systems, field studies of diel cycling were conducted in two streams (Silver Bow Creek and High Ore Creek) affected by historical mining activities in southwestern Montana. A combination of sampling and modeling tools were used to assess the toxicity of metals in these systems. Diffusive Gradients in Thin Films (DGT) samplers were deployed at multiple time intervals during diel sampling to confirm that DGT integrates time-varying concentrations of dissolved metals. Thermodynamic speciation calculations using site specific water compositions, including time-integrated dissolved metal concentrations determined from DGT, and a competitive, multiple-metal biotic ligand model incorporated into the Windermere Humic Aqueous Model Version 6.0 (WHAM VI) were used to determine the chemical speciation of dissolved metals and biotic ligands. The model results were combined with previously collected toxicity data on cutthroat trout to derive a relationship that predicts the relative survivability of these fish at a given site. This integrative approach may prove useful for assessing water quality and toxicity of metals to aquatic organisms in dynamic systems and evaluating whether potential changes in environmental health of aquatic systems are due to anthropogenic activities or natural variability.

**RP014 Influence of food quantity on fathead minnow (*Pimephales promelas*) reproduction and metal accumulation during exposure to metal mine effluent** J.D. Ouellet, University of Saskatchewan / Biology; M. Dube, Total E&P Canada Ltd; S. Niyogi, University of Saskatchewan / Department of Biology. Treated metal mine effluents (MMEs) are discharged into waterways. Some of these mine effluents have been shown to reduce invertebrate densities and fathead minnow (*Pimephales promelas*) egg production during chronic exposures, however it is often unknown which factors cause effects. Traditionally, metal contamination was believed to be the main factor in reducing both chironomid densities and fathead minnow egg production. This study examined whether the quantity of *Chironomus dilutus* available as food could also influence fathead minnow egg production during a chronic exposure to a treated MME. Breeding pairs of fathead minnows were exposed to either reference water or MME and fed 0.4 g (low food) or 1.2 g (high food) daily for 21 days during which egg production was monitored. Reference fish were fed *C. dilutus* cultured in reference water while MME exposed fish were fed *C. dilutus* cultured in MME. We observed significantly greater egg production, gonad sizes, liver sizes, and body sizes in the high food reference and MME fathead minnows relative to the low food reference and MME fathead minnows (Two-way ANOVA;  $P \leq 0.05$ ). Metals concentrations in the livers were generally increased in the low food MME treatment relative to all other treatments (Two-way ANOVA;  $P \leq 0.05$ ). This suggested that although fathead minnows under high food MME conditions were exposed to increased metal concentrations through the diet, they were still able to eliminate these metals more effectively than fish under low food MME conditions. For the majority of metals that were examined, food quantity was found to be the primary factor in influencing tissue metal concentrations (e.g., liver, gonads, gills, and carcass). Tissue concentrations of Rb, Se, Tl, Ni, and Fe, however, were generally dependent on either the MME or both the food quantity and MME. Overall, our results suggest that food quantity influenced egg production, fish morphometrics, and tissue metal concentrations, while the presence of MME alone was not a major factor in decreasing fathead minnow egg production. These results are significant as they suggest that any environmental contaminant that reduces *C. dilutus* densities might also therefore indirectly contribute to decreases in fathead minnow egg production and/or elevated contaminant concentrations in fish tissues.

**RP015 Influence of test conditions and bioturbation on toxicity of nickel-spiked sediments to burrowing mayflies (*Hexagenia* sp.)** J. Besser, U.S. Geological Survey / Columbia Environmental Research Center, USGS / Columbia Environmental Research Center; W. Brumbaugh, USGS / Columbia Environmental Research Center; C. Ingersoll, USGS. Sediment quality guidelines for metals, such as the USEPA's Equilibrium-Partitioning Sediment Benchmarks (ESBs), are largely based on toxicity data from a few standard test organisms, but few studies have examined whether these models predict toxicity across a wide range of benthic taxa. We conducted chronic whole-sediment toxicity tests with eight species of benthic invertebrates and eight nickel-spiked sediments with a broad range of acid volatile sulfide, total organic carbon, and other characteristics. Results of the first round of tests with 8 taxa (2 amphipods, 2 midges, 1 mayfly, 2 oligochaetes, 1 mussel) and 2 sediments (low AVS and TOC; high AVS and TOC) generally agreed with ESB models, with toxicity predicted by nickel concentrations expressed as either [SEM-AVS] or pore-water nickel. The most sensitive taxa -- two amphipods (*Hyalella* and *Gammarus*) and a burrowing mayfly (*Hexagenia*) -- were included in a second round of tests with six additional sediments. Toxicity of nickel-spiked sediments to both amphipods corresponded closely to predictions of ESB models based on [SEM-AVS], [SEM-AVS]/ $f_{\text{oc}}$ , or pore-water nickel, but toxicity to *Hexagenia* did not. Nickel toxicity to *Hexagenia* (EC20s for reduced growth) differed between the first and second round of tests, reflecting differences in the size of nymphs tested and differences in chamber size, sediment depth, and sediment surface area. In the second round of tests, *Hexagenia* EC20s had a strong positive correlation with AVS, but the slope of the linear regression was much less than 1.0 and toxicity occurred in several sediments with [SEM-AVS] < 0 and with measured pore-water nickel concentrations less than water-only EC20s. Although nickel concentrations in mayfly burrows were not determined, oxidation of AVS-rich sediments on the walls of burrows could have caused the release of toxic concentrations of dissolved nickel into burrow microenvironments. Measured concentrations of pore-water nickel were not increased nor were AVS concentrations markedly decreased in *Hexagenia* test chambers. However, release of nickel to overlying water



was 50-90% greater in *Hexagenia* tests compared to tests with other taxa. These results suggest that localized oxidation of AVS caused by bioturbation can result in toxic effects on *Hexagenia* in nickel-contaminated sediments that are predicted by ESB models to be non-toxic.

**RP016 Trophic structure and metal bioaccumulation differences in multiple fish species exposed to coal ash-associated metals** R.R. Otter, Middle Tennessee State University / Biology, Middle Tennessee State University / Department of Biology, Middle Tennessee State University / Assistant Professor; F.C. Bailey, Middle Tennessee State University / Department of Biology; A. Fortner, Arcadis US; M. Adams, Oak Ridge National Laboratory. On December 22, 2008 a dike containing coal fly ash from the Tennessee Valley Authority Kingston Fossil Plant near Kingston Tennessee USA failed and resulted in the largest coal ash spill in U.S. history. Coal ash, a by-product of coal combustion, is known to contain multiple contaminants of concern, including arsenic and selenium. The purpose of this study was to investigate species differences in the bioaccumulation of arsenic and selenium and potential factors contributing to these differences (i.e., trophic dynamics and gut pH) in the vicinity of the Kingston coal ash spill. Elevated levels of arsenic and selenium were observed in various tissues of largemouth bass, white crappie, bluegill and redear sunfish from sites associated with the Kingston coal ash spill. Highest concentrations of selenium were found in redear sunfish with liver concentrations as high as 24.83 mg/kg dry weight and ovary concentrations up to 10.40 mg/kg dry weight at coal ash-associated sites. Investigations into the gut pH and trophic dynamics of redear sunfish and bluegill demonstrated a large difference in the gut physiology between these two species. Redear sunfish stomach and intestinal pH was found to be 1.1 and 0.16 pH units higher than in bluegill, respectively. In addition, fish from coal ash-associated sites showed enrichment of  $^{15}\text{N}$  &  $^{13}\text{C}$  compared to no ash sites, indicating differences in food web dynamics between sites. These results imply the incorporation of coal ash-associated compounds into local food webs and/or a shift in diet at ash sites compared to the no ash reference sites.

**RP017 Uranium Exposure and Toxicity Modifying Factors** M. Trenfield, Environmental Research Institute of the Supervising Scientist, Darwin, Australia & The University of Queensland, National Research Centre for Environmental Toxicology, Coopers Plains, Australia / Department of Environment, Water, Heritage & the Arts; R. van Dam, Environmental Research Institute of the Supervising Scientist, Darwin, Australia; P.M. Chapman, Golder Associates Ltd.. Water quality guidelines (WQGs) are often developed without considering exposure and toxicity modifying factors (ETMFs) that occur in the environment. This is largely because ETMFs typically are not included in the laboratory toxicity testing used to derive such "default" WQGs. Consequently, default WQGs often have a very high level of conservatism and, thus, a low level ability to discriminate contaminant concentrations of no concern from those requiring additional investigation. Consideration of ETMFs maintains conservatism while improving discriminatory ability. Biotic ligand models represent the most advanced way to date of accounting for ETMFs in WQGs, but have been developed for only a few metals and aquatic species. One compound for which ETMFs have not yet been incorporated into WQGs is uranium. Robust but highly conservative WQGs for uranium were published in Canada in 2011. At the time, however, there were insufficient data to enable the incorporation of ETMFs, despite evidence that a number of ETMFs (e.g., pH, dissolved organic carbon [DOC], hardness, alkalinity) were influential for uranium. Recently, the effect of DOC on uranium bioavailability and toxicity has been quantified, resulting in the development of an algorithm to modify WQGs depending on DOC concentration. Whilst this is a positive first step, the effects of other important uranium ETMFs, in particular, pH, need to be further quantified such that they can be incorporated into both generic WQGs and site-specific water quality benchmarks. The implications of uranium ETMFs to regulatory permitting, effluent management or treatment, and decommissioning will be discussed.

**RP018 A Systems Toxicology Approach to Elucidate the Mechanisms Involved in RDX Species-Specific Sensitivity** K.A. Gust, ERDC-EL-EP-P / Environmental Laboratory, US Army, Engineer Research and Development Center, US Army Engineer Research & Development Center, US Army Engineer Research and Development Center, ERDC-EL-EP-P; C. Warner, Keck Graduate Institute; J.K. Stanley, U.S. Army Engineer Research and Development Center / Environmental Laboratory, U.S. Army Engineer

Research And Development Center / Environmental Laboratory, U.S. Army Engineer Research and Development Center / CEERD-EP-R; T. Habib, BTS; M. Wilbanks, US Army Engineer Research and Development Center; N. Garcia-Reyero, Mississippi State University / Institute for Genomics Biocomputing and Biotechnology; E.J. Perkins, US Army Engineering Research & Development / Environmental Laboratory, US Army ERDC / Environmental Processes & Effect Division, US Army Engineering Research & Development / Environmental Genomics and Genetics Team. Inter-species uncertainty factors in ecological risk assessment provide conservative estimates of risk where limited or no toxicity data is available. We quantitatively examined the validity of inter-species uncertainty factors by comparing the responses of zebrafish (*Danio rerio*) and fathead minnow (*Pimephales promelas*) to the energetic compound 1,3,5-trinitroperhydro-1,3,5-triazine (RDX), a known neurotoxicant. Relative toxicity was measured through transcriptional, morphological, and behavioral endpoints in zebrafish and fathead minnow fry exposed for 96h to RDX concentrations ranging from 0.9 to 27.7 mg/L. Spinal deformities and lethality occurred at 1.8 and 3.5 mg/L RDX respectively for fathead minnow and at 13.8 and 27.7 mg/L for zebrafish, indicating that zebrafish have an eight-fold greater tolerance for RDX than fathead minnow fry. The number and magnitude of differentially expressed transcripts increased with increasing RDX concentration for both species. Differentially expressed genes were enriched in functions related to neurological disease, oxidative-stress, acute-phase response, vitamin / mineral metabolism and skeletal / muscular disorders. Decreased expression of collagen-coding transcripts were associated with spinal deformity and likely involved in sensitivity to RDX. Our work provides a mechanistic explanation for species-specific sensitivity to RDX where zebrafish responded at lower concentrations with greater numbers of functions related to RDX tolerance than fathead minnow. While the 10-fold interspecies uncertainty factor does provide a reasonable cross-species estimate of toxicity in the present study, the observation that the responses between ZF and FHM are markedly different does initiate a call for concern regarding establishment of broad ecotoxicological conclusions based on model species such as zebrafish.

**RP019 Androgen receptor mediated compensation of estradiol in response to aromatase inhibition: a mathematical model** M. Mayo, US Army ERDC; T. Habib, BTS; N. Garcia-Reyero, Mississippi State University / Institute for Genomics Biocomputing and Biotechnology; D.L. Villeneuve, U.S. EPA / National Health and Environmental Effects Research Laboratory, U.S. EPA; G.T. Ankley, US EPA / Mid-Continent Ecology Division; E.J. Perkins, US Army Engineering Research & Development / Environmental Laboratory, US Army ERDC / Environmental Processes & Effect Division, US Army Engineering Research & Development / Environmental Genomics and Genetics Team. Chemicals in the environment have the potential to cause reproductive toxicity by acting on the hypothalamus-pituitary-gonadal (HPG) axis. We have developed a mathematical model to predict chemical impacts on reproductive hormone production in the highly conserved HPG axis using the fathead minnow (*Pimephales promelas*). The HPG axis controls the supply of reproductive hormones available to the body through feedbacks between local hormone levels and enzymes produced in response to changes in gene expression. This behavior has been recently observed in studies of fathead minnow exposed to the highly selective aromatase inhibitor fadrozole. In these studies, the enzyme-mediated transformation of testosterone into estradiol, strongly correlated with egg production (oogenesis), is initially inhibited by fadrozole. However, over time direct inhibition of aromatase by fadrozole is offset, by induction of several central steroidogenic (cytochrome p450) enzymes critical to estradiol biosynthesis. The biochemical mechanisms mediating this apparent compensatory gene expression have not been fully characterized. However, microarray-based gene expression studies conducted by our group suggest the androgen receptor mediates may mediate these compensation pathways. Here we develop a mechanistic mathematical model, grounded in experimental data, to test these compensation hypotheses. Parameterized using data primarily from qPCR and literature sources, this model reproduces gene-based induction events that drive *de novo* aromatase production and associated increases in estradiol production at lower fadrozole doses (3 ug/L), but predicts decreased reduced capacity for compensation at higher doses (30 ug/L) despite further gene expression increases, consistent with current experiments. Extensions of this predictive model to other less selective aromatase inhibitors (e.g., ketoconazole) will be discussed.

**RP020 Drivers of Contaminant Variability in Fish Communities** A. McLeod, D. Haffner, K. Drouillard, GLIER. Understanding the individual variation of contaminants is critical for quantifying hazards of contaminants to aquatic organisms and for the establishment of fish consumption guidelines. Until recently, most variation within these studies was largely neglected or treated as statistical noise. For instance, models of contaminant accumulation simulate the chemical body burden of the average fish, failing to incorporate the inherent inter-individual variation present in natural populations. Polychlorinated Biphenyls (PCBs) have been recognized as persistent, toxic chemicals since the 1970s and decades of research have gone into understanding and quantifying their accumulation in aquatic ecosystems. Here, I examine the possible drivers of variability for congeners spanning a range of hydrophobicities. I compare individual variability of PCBs both among sites and species to determine the relative contributions of ecological and chemical drivers highlighting the importance of water exposure, sediment exposure, and diet. Finally, I will demonstrate that quantitative ecological tracers can be chosen to investigate differences in feeding ecology and bioaccumulation between species.

**RP021 Hemocytic lysosome response in the mussel *Mytilus galloprovincialis* after exposure to Tributyltin** H.K. Okoro, Cape Peninsula University of Technology / Faculty of Applied Sciences; R. Snyman, O. Fatoki, B. Ximba, Faculty of Applied Science, Cape Peninsula University of Technology; F. Adekola, Department of Chemistry, Faculty of Science, University of Ilorin. This study aimed to use responses of lysosomal membranes of hemocytes of the mussel, *Mytilus galloprovincialis*, as a biomarker of stress due to exposure to tributyltin. The neutral red retention time assay was employed for this purpose. Mussels are known to accumulate metals and to destabilize lysosomal membranes, eventually resulting in cell death. The neutral red retention time assay is a common technique to measure temporal changes in lysosomal membrane integrity and the latter has been identified as an early warning system for metal-induced stress. Two groups of mussels were exposed to different environmentally relevant concentrations of tributyltin (as measured in the Cape Town harbour, South Africa). A third group served as control. The experiment ran over four weeks. Each week, hemolymph was drawn from 6 animals per group, the neutral red vital dye added and the numbers of stained and unstained cells counted, at two-minute intervals, using a compound microscope. The point at which 50% of the cells were stained, was taken as the neutral red retention time. Subsequent to cell counts, the animals were used for TBT analysis by methanolic acid digestion method with in-situ derivatization by 1% Sodiumtetraethylborate ( $\text{NaTEb}_4$ ) using GC-FPD. Detailed results regarding retention times, over the four-week period, and their relationship with TBT bioaccumulation, will be discussed. Conclusions will be drawn regarding the usefulness of the measured responses as biomarker of TBT induces stress in this species.

**RP022 Structure toxicity of alkyl-substituted polycyclic aromatic hydrocarbons (PAHs) to Japanese medaka embryos (*Oryzias latipes*)** H. Lin, Queen's University, Queens University. Alkylated polycyclic aromatic hydrocarbons (PAHs) are a suite of compounds predominating in crude oils. Most research on alkyl PAHs has focused on alkyl-phenanthrenes, especially retene (7-isopropyl-1-methyl-phenanthrene), but there is little research on other three or more ringed alkyl PAHs. The alkylation at different locations on the benzene rings of the PAH changes the size and shape, which could result in different interaction with tissue receptor and different severities of toxicity. This project will assess the toxicity of chrysene, benzo(a)anthracene, and their derivatives to the embryos of Japanese medaka (*Oryzias latipes*) using the partition controlled delivery (PCD) method of exposure. With PCD, a desired test concentration is maintained by equilibrium partitioning of alkyl-PAHs from polydimethylsiloxane (PDMS) films. I proposed to develop a structure-toxicity relationship between the measured toxicity and some physical or chemical properties of the group of alkyl-PAHs. The occurrence of blue sac disease (BSD) as a classic sign of embryo toxicity will be recorded. Thereafter, median effective concentration ( $\text{EC}_{50}$ ), the representation of the severity of toxicity of each alkyl-PAH, could be predicted. The octanol water partition coefficient ( $K_{ow}$ ) is one representation of physical and chemical property that describes the relative lipid solubility of compounds and the propensity for bioaccumulation. A structure-toxicity association could be illustrated by the  $\log\text{EC}_{50} - \log K_{ow}$  function.

**RP023 The fitting model of hormesis and its toxicity mechanism based on quorum sensing: a case study on the toxicity of sulfonamides to *P. phosphoreum*** Z. Lin, Tongji University / College of Environmental Science and Engineering; D. Wang, Tongji University / College of Environmental Science and Engineering; Z. Yao, Z. Deng, D. Yin, Tongji University. During the past two decades, the phenomenon of hormesis has gained increased recognition. However, mechanistic understanding of hormesis by far is extremely limited. A model that have mechanistic basis and provide benefit for mechanism study is strongly needed. Here, we propose a parametric model for hormetic dose-response, which decompose the biphasic dose-response into two separate parts: stimulation and inhibition. Based on new model, two parameters describing hormesis were further proposed, which were found to be convenient to access and consistent with the former used ones. The new model and parameters was applied to the toxicity results of sulfonamides on *Photobacterium phosphoreum*. Based on the approach of Quantitative Structure Activity Relationships (QSARs) and molecular docking, the mechanism of toxicity was explained to the level of receptors. A mechanistic explanation for hormesis was proposed by introducing the concept of quorum sensing into toxicological study for the first time. According to the explanation, the low dose sulfonamides bind to LuxR, the receptor of autoinducer, and promote the express of *luxR* genes. As the cell density increase, largely synthesized autoinducer bind to the accumulated LuxR, causing the bacteria activate quorum sensing earlier, thus produce more light than control and exhibits a stimulation effect. This mechanistic theory, explained both the dose-dependent and time-dependent features of hormesis, along with the new model, could give a new insight into the mechanistic study of hormesis.

**RP024 Occurrence of PAHs and n-alkanes in soil of the Pearl River Delta, South China with special reference to anthropogenic impact** Y. Wei, C. Wu, J. You, Guangzhou Institute of Geochemistry, Chinese Academy of Sciences; E.Y. Zeng, Guangzhou Institute of Geochemistry / State Key Lab of Organic Geochem. The Pearl River Delta PRD of South China has experienced rapid economic growth and urbanization in the last 30 years. To evaluate the impact of anthropogenic events on the environment, a comprehensive sampling campaign was conducted from December 2009 to March 2010 to collect soil samples analyzed for polycyclic aromatic hydrocarbons PAHs and n-alkanes. The PRD region was divided into six land use types, i.e., industrial, agricultural, fontinal, landfill, residential and forest, and mapped with equally sized hexagons as sampling sites (at approximately the center of each hexagon). The concentrations of the sums of 25 n-alkanes ( $\Sigma_{25}\text{n-alkane}$ ), 28 PAHs ( $\Sigma_{28}\text{PAH}$ ), and 15 PAHs ( $\Sigma_{15}\text{PAH}$ ) were in the ranges of 73–9,400, 10–21,000, and 7.2–12,000 ng/g dry wt., respectively. There were no significant differences of  $\Sigma_{25}\text{n-alkane}$ ,  $\Sigma_{27}\text{PAH}$  and  $\Sigma_{15}\text{PAH}$  concentrations among different land use types, with some sampling sites showing very high analyte concentrations. The concentration distributions of  $\Sigma_{25}\text{n-alkane}$ ,  $\Sigma_{28}\text{PAH}$ , and  $\Sigma_{15}\text{PAH}$  were analyzed using kriging interpolation in Geographic Information System (GIS). The results indicated that higher concentrations occurred in the center of the PRD, e.g., Guangzhou, Shenzhen, and Dongguan with higher population density than other regions, reflecting the impact of anthropogenic events on the environment. On the other hand, the level of organic pollution in soil of the PRD appeared to be moderate compared to other regions in China and around the world. For example, the median concentration (110 ng/g dry wt.) of  $\Sigma_{15}\text{PAH}$  in the PRD was comparable to those in other cities of China, such as Hong Kong (140 ng/g), Shanghai (314 ng/g), and Harbin (301 ng/g), but lower than those obtained in other highly industrialized cities around the world, i.e., Torino of Italy (704 ng/g), Glasgow of Scotland (8,337 ng/g), and Ljubljana of Slovenia (791 ng/g).

**RP025 Computational Study of the Thermodynamics of Atmospheric Nitration of PAHs via OH-Radical-Initiated Reaction** N. Jariyasopit, Oregon State University / Department of Environmental & Molecular Toxicology; P. Cheong, Oregon State University / Chemistry; S. Simonich, Oregon State University / Depts. of Chemistry and Environmental & Molecular Toxicology. Nitrated polycyclic aromatic hydrocarbons (NPAHs) are an important class of PAH derivatives that are more toxic than their parent PAHs and are emitted from direct emission and secondary emission to the atmosphere. The secondary emissions, particularly the OH-radical initiated and  $\text{NO}_3$ -radical-initiated reactions, have been shown to influence the NPAH concentrations in the atmosphere. Gas-phase reactions are thought to be the major sources of NPAHs containing four or fewer rings. Besides



NPAHs, PAHs lead to a number of other products including oxygenated, hydroxy substituted and ring-opened PAH derivatives. For some PAHs, the OH-initiated and NO<sub>3</sub>-initiated reactions result in the formation of different NPAH isomers, allowing the ratio of these isomers to be used in the determination of direct or secondary emission sources. Previous studies have shown that the PAH gas-phase reactions with OH radical is initiated by the addition of OH radical to the aromatic ring to form hydroxycyclohexadienyl radicals. In the presence of NO<sub>2</sub>, these reactive intermediates readily nitrate with the elimination of water. The hydroxycyclohexadienyl-type radical intermediates are also prone to react with other species in the atmosphere or revert back to the original compound. The objective of this study was to investigate the thermodynamics of PAH nitration through day-time OH-radical-initiated reactions. The theoretical investigation was carried out using Density Functional Theory (B3LYP) and the 6-31G(d) basis set, as implemented in Gaussian03. A number of different PAHs were studied including fluoranthene, pyrene, as well as the molecular weight 302 PAHs such as dibenzo[a,l]pyrene. Computations were also used to predict unknown NPAHs formed by OH-radical-initiated reaction. All intermediates for the OH-radical addition and the following nitration were computed. We have discovered that the thermodynamic stability of the intermediates involved in the PAH oxygenation and nitration pathways are critical in explaining the atmospheric abundances of NPAHs. Specifically, we have found that the experimentally most abundant species had the most stable intermediates. Interestingly, the overall free energy of reaction was not a factor in determining the relative abundances of NPAHs.

**RP026 Chlorinated Polycyclic Aromatic Hydrocarbons in Urban Surface Dust and Soil of Shanghai, China** J. Ma, Shanghai University, China; J. Zheng, Z. Chen, M. Wu, Shanghai University; Y. Horii, Center for Environmental Science in Saitama; T. Ohura, Institute for Environmental Science, University of Shizuoka; K. Kannan, Wadsworth Center, New York State Department of Health, and Department of Environmental Health Sciences, School of Public Health, State University of New York at Albany, Albany, New York. Chlorinated polycyclic aromatic hydrocarbons (ClPAHs) are a group of halogenated contaminants found in the urban environment with a toxic potential similar to dioxins. Information about the distribution and characteristics of ClPAHs in urban dust and soil is limited. In the present study, concentrations of 20 ClPAH congeners were measured in road dust and soil from a park and lake area, a chemical industrial complex, waste incineration power plants, a crossroads along arterial traffic, and a steel factory in Shanghai. ClPAHs were ubiquitous and log-normally distributed in road dust and soils with a range from 0.27 to 206 ng/g dw for dust, and with a range from 0.05 to 94.3 ng/g dw for soils. ClBaP was not the predominant analogue in all samples. Instead, ClPyr and ClPhe were the main analogues in road dust in the present study. It was inferred that the ClPAHs detected in the road dust samples originated from vehicle exhaust.

**RP027 Relationship between molecular descriptors and photochemical productions of halogenated polycyclic aromatic hydrocarbons on solid phase** K. Sankoda, Prefectural University of Kumamoto / Graduate School of Environmental and Symbiotic Sciences; K. Nomiya, Ehime University / Center for Marine Environmental Studies (CMES); R. Shinohara, Prefectural University of Kumamoto / Graduate School of Environmental and Symbiotic Sciences. The goal in this study was to demonstrate molecular descriptors, which associated with photochemically-induced production amounts of halogenated derivatives of polycyclic aromatic hydrocarbons (PAHs) in the environment. Six congeners of PAH, including two isomer pairs (phenanthrene and anthracene; fluoranthene and pyrene), were irradiated with the natural sunlight under simulated conditions of tidal flats, and 11 molecular descriptors were conducted based on PM6 Hamiltonian. In the product of 13 halogenated derivatives confirmed after irradiation of sunlight, the highest production amount was found in 6-chlorobenz[a]pyrene. Moreover, pyrene showed the behavior that produced halogenated derivatives significant strongly than its structural isomer of fluoranthene. After the determination, correlation between the production amounts of halogenated derivatives and the molecular descriptors of individual PAHs were examined. The energy of the highest occupied molecular orbital (E<sub>HOMO</sub>), the lowest unoccupied molecular orbital (E<sub>LUMO</sub>) and the energy gap between HOMO and LUMO (E<sub>HOMO-LUMO</sub>) showed significant correlation with the production amounts. In particular, E<sub>HOMO-LUMO</sub> was the most effective, and enough to explain differences in the production amounts among structural isomers. PAHs that have small E<sub>HOMO-LUMO</sub> showed high potentials to

produce halogenated derivatives. From these results, we concluded that E<sub>HOMO-LUMO</sub> of PAHs is an effective molecular descriptor to predict their photochemical behavior during sunlight irradiation.

**RP028 Probability of Human Exposure to 16 Priority Polycyclic Aromatic Hydrocarbons in Soils of Chattanooga, Tennessee, USA** S. Richards, University of Tennessee-Chattanooga / Department of Biological & Environmental Sciences; E. Hussar, Z. Lin, R. Dixon, K. Johnson, Southern Illinois University – Edwardsville. South Chattanooga had been home to foundries, coke furnaces, chemical, wood preserving, tanning and textile plants for over 100 years. Much of the associated waste was dumped into Chattanooga Creek and the floodplain. During the 1950s and 1960s, public housing was constructed in that floodplain. Polycyclic aromatic hydrocarbons (PAHs) comprised the large component of the waste. To investigate the probability of humans encountering soil with high concentrations of PAHs (as defined by EPA Residential Preliminary Remediation Goals (PRG)), a broad study of South Chattanooga soil was conducted on 20 sites across the city. Sixteen priority pollutant PAHs were quantified at two depths (0-10cm and 10-20cm) and compared against reference site soils, as well as to soils from industrially-impacted areas in Germany, China, and the US (e.g., New Orleans, LA, post Hurricane Katrina). We found that 40.0% and 35.0%, of sites in South Chattanooga had soil samples with ΣPAHs exceeding Bayreuth, Germany, and Beijing, China's highest residential ΣPAHs soil concentrations. Accordingly, 36.6%, 56.6% and 53.3% South Chattanooga's sites had higher concentrations of B[a]A, B[b]F, and B[a]P (respectively) than the highest values found in New Orleans. From these data and corresponding Toxic Equivalency Factors (TEFs), the probability that people would encounter levels exceeding EPA Residential Preliminary Remediation Goals (PRG) was calculated. Results indicate that South Chattanooga soils have relatively high concentrations of total PAHs, specifically Benzo[a]pyrene (B[a]P). The geometric mean of ΣTEFs across South Chattanooga was 1.6 mg/kg soil with a range of < 0.05- to 113.9 mg kg<sup>-1</sup> soil. The geometric mean in South Chattanooga is comparable to the geometric means of other cities; however, the values in the upper range are considerably higher for the ΣTEFs in the soils from the present study. The ΣTEFs were largely a factor of the B[a]P concentrations. Indeed, high concentrations of B[a]P were somewhat ubiquitous in South Chattanooga. There is a high probability (88%) of encountering soil in South Chattanooga that exceeds the EPA PRG for B[a]P. However, there is a low probability (15%) of encountering a site with ΣPAHs exceeding EPA PRG guidelines. While the direct health effects associated with these encounters is very difficult to address, it is apparent that, in comparison to other industrially affected areas, PAHs are elevated across South Chattanooga.

**RP029 PAH Mixtures: Additivity, Synergism or Antagonism?** B.H. Magee, ARCADIS. USEPA's Polycyclic Aromatic Hydrocarbon (PAH) Mixtures Assessment is due to be issued by EPA in final form in 2012. In this document, EPA will issue the Relative Potency Factors (RPFs) that it has derived assuming that PAHs in mixtures act with the same mode of action as benzo(a)pyrene (BaP) and in an additive fashion. In addition, the possibility that PAHs may act synergistically is a reason often cited for the concerns that PAH risks are not being adequately evaluated with the current analytical methods and toxicological reference values. This presentation will summarize the results of a literature search and critical evaluation of toxicological tests with multiple PAHs tested alone and in mixtures. Studies demonstrating additivity, synergism or antagonism will be summarized. The research to date demonstrates that the weight of evidence of the scientific literature is that antagonism is far more commonly seen in such mixture tests than is additivity or synergism. This conclusion challenges the basic assumption of the RPF approach to assessing PAH mixtures in the environment.

**RP030 Bioavailability variation of sub-lethal PAH concentrations in three sediment types; consequences on meiofaunal- and microbial communities** F. Lindgren, I. Hasselov, Chalmers University of Technology / Department of Shipping and Marine Technology; I. Dahllof, University of Gothenburg / Department of Biological and Environmental Sciences. It was shown in a previous study (Lindgren et al., 2012) that PAH concentrations as low as 1300 µg/kg (dw) sediment after 60 days produced effects in both meio- and microbial fauna; with the results of lowered nitrification processes, reduction in silicate and phosphate fluxes indicating a lowered activity in the sediment, and an altered meiofaunal community composition. These effects became more distinct and increased over time during



the experiment. Same amount of PAHs added to different sediments types, with different characteristics, might produce dissimilar effects in meio- and microbial communities, more or less severe, due to different bioavailability conditions between the sediments. It is known that for example grain-size and organic carbon content affect the bioavailability of PAHs in sediment. This fact makes it important to consider whether sediment characteristics needs to be taken into account when estimating effects of small scale oil (PAH) discharges in for example risk assessment studies. A new study has therefore been performed where the same amount of PAHs, 1300 µg/kg (dw) sediment, were added to three different types of sediment through a weathering process in an experimental setup with continuous flow of seawater. Muddy sediment with very fine particles, sandy sediment with calcareous debris and sediment with high organic carbon content were sampled in the vicinity of the Gullmar Fjord, Sweden and used in the experiment. Sampling of meiofaunal densities, microbial fluxes, potential nitrification and potential denitrification were made after thirty and sixty days and were analysed. Preliminary results indicate an affected microbial nitrogen cycle in all sediment treatments and less affected meiofaunal communities by the PAH addition. Some alterations in the degree of effect between the different sediment types could be detected, with less severe effects in the muddy sediment with very fine particles possibly indicating less bioavailability of PAHs in this treatment. More detailed results will be presented at the conference.

**RP031 Interrelationship of Polycyclic Aromatic Hydrocarbons in Sediment Cores from Sub-Tropical Region with Algal-derived Organic Matter and Global Change** D. Duan, Guangzhou Institute of Geochemistry / State Key Laboratory of Organic Geochemistry; Y. Ran, Guangzhou Institute of Geochemistry / Chinese Academy of Sciences. The study investigates the influence of algal-derived organic matter and global change on the historical accumulation of polycyclic aromatic hydrocarbons (PAHs) in the sediments of three reservoirs with different tropic levels from the Pearl River Delta (PRD). Up to our knowledge, this investigation is for the first time conducted in the sub-tropical and the tropical regions although some previous investigations were conducted in the Arctic and high-altitude regions. The 16 US EPA priority PAHs were measured by gas chromatography coupled with mass spectrometer (GC-MS), the algal organic matter in the sediment cores were investigated by the Rock-Eval analysis. The stable metal titanium (Ti) was also determined by inductively coupled plasma mass spectrometry (ICP-MS) to ascertain the hydrological and sedimentary variations in the lakes. It was found that the sedimentary concentrations of PAHs increase as the sedimentary depth decreases, and are very significantly related to the algal-derived organic matter in the sediments of the Zengtang and the Lian'an reservoirs, which respectively represent a eutrophic lake and a meso-eutrophic lake, even though the atmospheric concentrations of PAHs in those areas have declined. For the Xinfengjiang reservoir representing an oligotrophic lake, the sedimentary concentrations of PAHs decrease as the sediment depth decrease, and are also significantly related to the algal-derived organic matter in the sediment. Moreover, it was observed from the historic climate records that the annual mean atmospheric temperature in this region increases by 2-3 degrees during the last 50 years. Therefore, these findings suggest that the algal productivity recorded in the sedimentary cores in the eutrophic and meso-eutrophic lakes from the subtropical and tropical regions such as the PRD are also coupled with the global climate change. However, as the organic matter in the sedimentary core of the oligotrophic lake had experienced oxidation and degradation, it can not reflect the algal productivity and then the global climate change. The above result also implies an overestimation of the historical record of atmospheric deposition of PAHs preserved in the sediment cores in eutrophic lakes. It is noted that the air-water interface input of PAHs in the oligotrophic lake is affected to the less extent than in the eutrophic or meso-trophic lake.

**RP032 Polycyclic Aromatic Hydrocarbons (PAHs) in Surface Sediment, Oysters and Mussels from Colombia Harbors** B. Johnson-Restrepo, University of Cartagena / Chemistry in the Environment Group; G. Mejia-Monteros, University of Cartagena; H. Junca, Corporación CorpoGen. Tropical ecosystems are being polluted by anthropogenic sources in the Caribbean Coast, Colombia. Surface sediments, Oysters, and Mussel were collected from the most important harbors on the Caribbean Sea in Colombia: Cartagena, Barranquilla and Santa Marta. Polycyclic Aromatic Hydrocarbons (PAHs) were identified and analyzed using gas chromatography coupled with mass spectrometry (GC/MS). Sediment samples were submitted to Soxhlet extraction for 16 h using a hexane/dichloromethane

mixture. Extracts were clean-up by column chromatography packed with activated Florisil. Extracts obtained were concentrated by rotary evaporation and then further concentrated to 1 ml under a gentle nitrogen stream and stored in vial. Samples were measured in a gas chromatography (GC) Agilent Technologies 7890A equipped with a mass spectrometer (MS) Agilent Technologies 5975C series. A DB-5 fused silica capillary column coated with 5% phenyl methyl polysiloxane (30 m length 0.25 mm i.d. 0.25 µm film thickness; J&W Scientific, Folsom, CA) was used. The column oven temperature was programmed from 80 °C (1 min hold) to 100 °C at a rate of 25 °C/min, and then ramped at a rate of 5 °C/min to 300 °C, with a final holding time of 6 min. The injector and detector temperatures used for this analysis were maintained at 250 °C and 300 °C, respectively. The total concentrations of 16 US EPA priority pollutant PAHs for surface sediments ranged from ND to 237 ng/g dw, while for Oysters ranged from ND to 3.3 ng/g dw and for mussel ranged from ND to 127.5 ng/g dw. Despite the great concentrations differences, the composition of PAHs in the sediments of the harbors were similar patterns dominated by PAHs with three or more rings. Distribution patterns of PAHs were examined by multivariate analysis approach indicating the results that the major PAHs contribution to pollution in the harbor sediments for PAHs were pyrogenic sources.

**RP033 Quantification of Polycyclic Aromatic Hydrocarbon Mixtures in Standard Reference Materials Using GC×GC/ToF-MS** C. Manzano, Oregon State University / Department of Chemistry; E. Hoh, San Diego State University / Graduate School of Public Health; S. Simonich, Oregon State University / Depts. of Chemistry and Environmental & Molecular Toxicology. Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous compounds in the environment and originate from incomplete combustion of organic matter during energy and industrial processes. PAH derivatives can also be formed as byproducts of transformation reactions in the environment. Identifying and quantifying PAHs in environmental samples is of concern due to the carcinogenic and mutagenic properties of some isomers. Some PAH derivatives, such as 1-Nitropyrene, have higher mutagenic and carcinogenic potential than its parent PAH. Other PAH derivatives, such as halogenated are being studied due to their potential persistence, bioaccumulation, and toxic effects. The objective of this research was to develop a single analytical method to identify and quantify complex mixtures of PAHs, including alkyl-, nitro-, oxy-, thio-, chloro- and bromo-PAHs present in two standard reference materials (NIST-SRM1975: diesel extract, and NIST-SRM1650b: Diesel particulate matter) in a single chromatographic analysis. A method based on comprehensive two-dimensional gas chromatography/time of flight mass spectrometry (GC×GC/ToF-MS) was developed and used. Two column combinations were tested, combination "A": 30m Rxi-5ms column in the first dimension followed by a 1.2m Rtx-17 column in the second dimension, and combination "B": 10m LC-50 liquid crystal column in the first dimension followed by a 1.2m NSP-35 nano-stationary phase in the second dimension. Combination "B" showed greater orthogonality and separation from interferences and unresolved complex mixture (UCM) in a previous study. Five-point calibration curves were developed for a standard mixture of a total of 93 different PAHs, with an average R<sup>2</sup> of 0.994 in for both column combinations. The quantitation was performed using the three highest modulated peaks for each compound and the ratio of internal standard to target in order to decrease the amount of error associated with quantitation processes in two-dimensional GC methods. For both column combinations, the results obtained corresponded well to the certified values for both standard reference materials within a 95% confidence interval, showing that combination "B" can provide good chromatographic separation and reliable quantification of complex mixtures of PAHs in environmental samples.

**RP034 Detection of PAHs using LC-APPI-MS/MS assisted by dopant gradient system** C. Wang, Florida International University / Chemistry Department; C.E. Ramirez, Florida International University / Ph.D. Student; P. Gardinali, Florida International University / Department of Chemistry and Biochemistry. This study demonstrated a dopant gradient deliver system for the detection of polycyclic aromatic hydrocarbon (PAHs) using high performance liquid chromatography atmospheric photoionization tandem mass spectrometry (HPLC-APPI-MS/MS). Five PAHs including naphthalene, phenanthrene, chrysene, perylene and benzo(g,h,i) perylene were chosen as model compounds. Model compounds cover the aromatic ring numbers from 2 to 6. Thermo Scientific Hypersil Green PAH column (100 mm × 2.1 mm, particle size 3 µm) was used to separate the

five target compounds and 100% methanol was used as mobile phase at a flow rate of 500  $\mu\text{L}/\text{min}$ . The five target compounds were well separated and eluted in order of molecular weight. A TSQ quantum Access Tandem MS QqQ (Thermo Scientific, USA) was used as the detector, equipped with an APPI interface (Syagen, USA). Results showed that all 5 PAHs were ionized when chlorobenzene was used as dopant. However, anisole increased the signal intensity of perylene and benzo(g,h,i)perylene, whose intensity were improved to 120% and 140% respectively. Therefore, a dopant gradient system was created using two Fusion Touch syringe pumps (Chemx, USA) optimizing the signal for every compound. At the beginning of analysis, chlorobenzene was used as dopant for naphthalene, phenanthrene and crysene, then dopant was changed to anisole for the analysis of perylene and benzo(g,h,i) perylene. It is the first report of a dopant gradient system to assist the detection of PAHs using HPLC-APPI-MS/MS.

**RP035 Using silicone membranes to produce truly dissolved solutions of polyaromatic hydrocarbons (PAHs) used in toxicological testing** L. Alvarez-Fraga, C. Ramirez, Florida International University / Department of Chemistry & Biochemistry; B. Echols, Florida International University / Southeast Environmental Research Center (SERC); G. Rand, Florida International University / Department of Earth & Environment and SERC; A. Edgington, W. Stubblefield, Oregon State University / Department of Environmental and Molecular Toxicology; P.R. Gardinali, Florida International University / Department of Chemistry & Biochemistry and SERC. Assessing the toxicological properties of polyaromatic hydrocarbons, in particular high-molecular ones (HMW-PAHs), requires the preparation of aqueous exposure media of known PAH concentrations. Because of the low water solubility of HMW-PAHs, preparing solutions for exposure usually involves the use of a "carrier" solvent to facilitate solution preparation (e.g., methanol or acetone). For solutions with high ionic strength (open ocean water) precipitation of the test compound is possible even in the presence of a carrier solvent and the chemical analysis of the water sample will likely overestimate the actual concentrations that organisms are exposed to. A number of studies have explored the use of a solid "vehicle" to passively release the compound into the testing solution. This preparation method uses testing chambers that, are coated with a layer of polydimethylsiloxane (PDMS), a solution of the testing material is diffused into the silicone matrix and upon drying and washing the PDMS layer becomes a reservoir saturated with the target toxicant. The clear disadvantage of this system is the need for a dedicated container that may need to be discarded or thoroughly decontaminated on every use. This work provides an alternative use of PDMS by using a silicone membrane to create a "truly dissolved" solution by passive diffusion of the target PAH(s) into the aqueous phase. The method uses concentrated solutions of the analytes (100ppm) in methanol placed inside 10 cm silicone tubes (3.18mm OD x 1.59mm ID) as passive diffusers. The filled tube(s) are attached to a Teflon stir bar and placed in an aspirator bottle containing the aqueous solution to equilibrate. For the proof of concept, 11 PAHs, including parent and alkyl homologues covering the range of compounds prevalent in crude oil (2 to 5 ring) and selected sulfur containing PAHs were tested. Equilibration for all compounds occurred within 20-30 hours. Saturation was tested using multiple dosing devices (n=1 to 3). Solubilities through the silicone membranes were compared to the theoretical ones (SPARC) corrected for the ionic strength of the preparation media (30PSU) by using the Setschenow equation. All samples were analyzed by online-SPE UPLC/APPI-MSMS without sample preparation. A predictive model in the form of  $\text{Log } S (\text{SPARC}) = 1.593 \text{ Log } S (\text{PDMS}) + 1.852$  was used to predict the "equilibrium" dissolved concentration of additional analytes and further validate the methodology.

**RP036 Comparison of Passive Sampling Devices (PSDs) for Measuring Dissolved PAH and oxy-PAH** K. O'Neal, NC State University / Department of Biology; W. Thorsen, P. Lazaro, X. Kong, X. Xia, North Carolina State University; D. Shea, North Carolina State University / Department of Biology. The measurement of trace concentrations (near or below 1 ng/L) of dissolved PAHs in water has always been a challenge, but passive sampling devices (PSDs) have shown promise to overcome this challenge. PSDs also have been useful in measuring PAHs in soil/sediment-water slurries to estimate the readily desorbable or exchangeable fraction of PAH. There also is interest in measuring degradation products of PAH using PSDs, but very little progress has been made to date. We will report on the equilibrium polymer-water partition coefficients and kinetic sampling rates for over 50 PAHs and related heterocyclic compounds and for a few oxygenated

PAHs (oxy-PAH) for the following polymers: polyoxymethylene (POM), polydimethylsiloxane (PDMS) and polyethylene (PE). Our measured partition coefficients are in good agreement with previously published values for the 16 EPA Priority Pollutant PAH and extend the list to over 50 PAH and 5 oxy-PAH degradation products. We then used all three PSD designs to measure PAH in PAH-contaminated aquatic field samples and soil-water slurries. All three designs gave nearly equivalent results, but POM yielded the lowest variability and the least co-extractable chemicals and exhibited a particular advantage in the presence of oil because oil adhered more to the PE and PDMS than to the POM. We will summarize the utility of each PSD polymer to measure dissolved PAH in both the laboratory and in field samples and in both the presence and absence of oil.

**RP037 Determination of a Standard Reference Compound for Estimation of Biodegradation Rates** L.E Barber, SRC, Inc.; J.P. Hassett, SUNY College of Environ. Sci. & Forestry / Chemistry Department. Aerobic biodegradation data were examined to determine if a standard reference compound could be used to predict the aerobic biodegradation rate of aromatic hydrocarbons in environmental media. Existing biodegradation rate data, collected by SRC, Inc. from a large number of literature sources, were utilized in this study. The data included aerobic  $0^{\text{th}}$ - and  $1^{\text{st}}$ -order rate constants in soil, surface water, sediments, and aquifers for 23 common organic chemicals. Preliminary analysis of the data showed that a constant ratio may exist between the environmental degradation rate constants of various aromatic compounds. This could enable the prediction of the aerobic biodegradation rate constant of an aromatic compound based on the rate of a standard reference compound. For the purpose of the study, the SRC data collection was limited to  $1^{\text{st}}$  order rate constants of single-ring, aromatic compounds. The data were sorted numerically by rate constant and a ratio was calculated for each pair of chemicals. The ratio was then plotted against the rate constant of each compound. Preliminary efforts concluded that at higher degradation rates, a useful ratio may exist, as a constant value signifies that the ratio is independent of the biodegradation rates. While further analysis is necessary, these results indicate that toluene may serve as a standard reference compound for prediction of the aerobic biodegradation rates of single-ring, aromatic hydrocarbons.

**RP038 Cerium oxide nanoparticles enhances lipid peroxidation in the shoot of germinating rice seeds** C. Rico; M.I. Morales, The University of Texas at El Paso / Chemistry; J. Hong, The University of Texas at El Paso / Environmental Science and Engineering PhD Program; L. Zhao, A.C. Barrios, J.R. Peralta-Videa, J.L. Gardea-Torresdey, The University of Texas at El Paso / Chemistry. Cerium oxide nanoparticles ( $\text{CeO}_2$  NPs) elicit varying degree of toxicity in food crops. However, there are no reports on the effects of  $\text{CeO}_2$  NPs on rice, despite the fact that rice is one of the most widely grown food crops in the world. In this research project, rice seeds were germinated and grown for 10 days in  $\text{CeO}_2$  NPs suspension at 62.5, 125, 250 and 500 mg/L concentrations. The uptake of Ce, growth performance, stress levels (hydrogen peroxide and lipid peroxidation), and enzymatic antioxidant responses [catalase (CAT), ascorbate peroxidase (APOX), superoxide dismutase (SOD), dehydroascorbate reductase (DHAR) and glutathione reductase (GR)] in both root and shoot of the seedlings were analyzed. Results showed that Ce in rice tissues increased with increased NP concentrations, but no visible signs of toxicity were apparent in the seedlings. Biochemical assays and in vivo imaging of  $\text{H}_2\text{O}_2$  revealed that, relative to the control, the  $\text{H}_2\text{O}_2$  generation significantly reduced with the 62.5 and 125 mg/L treatments, but did not significantly change with the 250 and 500 mg/L  $\text{CeO}_2$  NP treatments, in both shoot and root. Enhanced lipid peroxidation was observed in the shoots of seedlings grown with 250 or 500 mg/L  $\text{CeO}_2$  NPs. With regards to the enzymatic antioxidant activities, the 500 mg/L  $\text{CeO}_2$  NP treatment upregulated the APOX and DHAR activities, but downregulated the CAT and GR activities, in the root. On the other hand, both the 250 and 500 mg/L  $\text{CeO}_2$  NP treatments upregulated the SOD, CAT and DHAR activities, but downregulated the APOX and GR activities, in the shoot. These findings demonstrate that  $\text{CeO}_2$  NPs at concentrations below 500 mg/L was not toxic to the roots, but at concentrations higher than 250 mg/L was toxic to the shoots of germinating rice seeds. Furthermore, the results illustrate that  $\text{CeO}_2$  NPs at 62.5 and 125 mg/L concentrations decreased the stress levels in rice seedlings.

**RP039 Cadmium (Cd) accumulation in two soybean (*Glycine max*) cultivars as influenced by humidity and pre-exposure to Cd** J. Kikkert, H. Renkema, University of Guelph; B. Hale, University of Guelph / School of Environmental Sciences. Cadmium (Cd) accumulation in plants is known to be somewhat dependent on water use by the plant, as it is very xylem-mobile, and there is some evidence that Cd phyto-toxicity is expressed as increased transpiration by plants. The external effect of humidity was separated from the internal effect of toxicity, on Cd accumulation by soybean, using pre-exposure to elemental Cd and short-term exposure to  $^{106}\text{Cd}$  under high and low relative humidity conditions. Two cultivars of soybean (*Glycine max* L. var. 'OAC Bayfield' and 'OAC Champion', known high and low bean-Cd accumulators, respectively) were grown in half strength Hoagland's solution for three weeks. During their growth, one-third of plants were exposed to  $10\text{ }\mu\text{g L}^{-1}\text{ Cd(NO}_3)_2$  in solution. At the end of three weeks, all plants were placed into a chamber of higher or lower relative humidity for 24 hours. While in the chambers, two-thirds of the plants, including some of those pre-exposed to Cd, were exposed to  $10\text{ }\mu\text{g L}^{-1}\text{ }^{106}\text{Cd}$  in solution. After 24 hours, plants were harvested and the tissue was separated and analyzed for total tissue elemental and isotopic Cd. In shoots, accumulated  $^{106}\text{Cd}$  was higher in plants pre-exposed to Cd and higher in OAC Bayfield plants compared to OAC Champion plants; relative humidity had no effect on  $^{106}\text{Cd}$  accumulation. Yet, whole plant accumulation of  $^{106}\text{Cd}$  was similar between the two varieties and pre-exposure treatments. As expected, total Cd was greatest in those plants pre-exposed to Cd in shoot tissue and whole plant tissue. The study suggests that at this early growth stage, humidity does not influence Cd translocation to the above ground tissue; rather pre-exposure to Cd increases Cd distribution to above ground tissue, an effect that is more pronounced in OAC Bayfield. Applications to risk management will be presented.

**RP040 Thallium (Tl) toxicity to radish (*Raphanus sativus*) and durum wheat (*Triticum turgidum*) grown in soil** J. Kikkert, University of Guelph; B. Hale, University of Guelph / School of Environmental Sciences. There are few data describing thallium (Tl) toxicity to plants grown in Tl contaminated soils. The study considers Tl toxicity to durum wheat (*Triticum turgidum* L. var. Kyle) and radish (*Raphanus sativus* L. var. sativus "Raxe") grown in four different agricultural field soils, to assess the link between plant toxicity and Tl availability in soil, as influenced by soil-contaminant aging and soil type. The bulk soils were amended with 0 to  $200\text{ mg kg}^{-1}\text{ TlNO}_3$  and leached to reduce the "salt-effect". A 14-day toxicity experiment for each plant species was conducted at one month after spiking. All amended soils were air-dried and aged for 11 months before the 14-day toxicity experiment was repeated. At one month and 11 months, total and extractable concentrations of Tl in soil were measured and plant toxicity endpoints of root mass (DM), shoot mass (DM), and shoot length were collected. In soil, regardless of the aging time, extractable Tl was greatest in light soils compared to heavier soils and there was a linear relationship between total and extractable soil Tl. At one month exposure, toxicity of Tl to plants was observed. For radish, plant growth endpoints decreased as soil Tl concentrations increased when grown in lighter textured soils, an effect that was not observed in the heavier textured soils. For wheat, plant growth endpoints decreased with increasing soil Tl concentrations regardless of the soil texture. Eleven month exposure analysis is pending and will be presented. Preliminary results suggest that 1) Tl availability in soil is not altered over time and is influenced by soil texture and 2) that physiological variability is important in assessing plant toxicity since the responses cannot be reduced to soil chemistry alone. Modeled regressions and regulatory conclusions will be presented.

**RP041 The response of three macrophytes to fomesafen and thiamethoxam in microcosms: The benefits of testing multiple species simultaneously** V. Sousa Oliveira, University of Lavras / Department of Soil Science; M. Hanson, University of Manitoba / Department of Environment and Geography, University of Manitoba / Faculty of Environment; K. Solomon, University of Guelph / School of Environmental Sciences, University of Guelph / Centre for Toxicology, School of Environmental Sciences; J. Bestari, University of Guelph / Centre for Toxicology, School of Environmental Sciences; J. Lima, University of Lavras / Department of Soil Science. The impacts of fomesafen (a herbicide) and thiamethoxam (an insecticide with plant growth promoter properties) on three macrophytes (*Lemna minor*, *Myriophyllum spicatum*, and *Elodea canadensis*) were assessed under semi-natural field conditions. Outdoor 12 000 L microcosms were

treated with 2.5, 12.5, and  $25\text{ }\mu\text{g L}^{-1}$  fomesafen ( $n=1$ ) and 5, 25, and  $50\text{ }\mu\text{g L}^{-1}$  thiamethoxam ( $n=1$ ), plus untreated controls ( $n=3$ ), and were monitored for up to 77 days. There was no discernible effect of exposure to fomesafen or thiamethoxam in either *L. minor* or *E. canadensis*. *Myriophyllum spicatum* did not show any obvious signs of toxicity as a result of exposure to thiamethoxam, though some growth enhancement was evident. However, *M. spicatum* wet and dry mass of both shoots and roots decreased significantly with increasing fomesafen concentration and duration of exposure. Our study supports the approach of testing multiple species simultaneously in order to characterize effects. By capturing a range of responses from plants with different physiologies and growth forms, uncertainty in the extrapolation of data, especially from the standard laboratory duckweed species, is reduced.

**RP042 Implementation of a Multi-pronged Strategy for Higher Tier Aquatic Plant Risk Assessments** H. Ochoa-Acuna, Purdue University / Veterinary Pathobiology, Principal Investigator / Global Regulatory Ecotoxicology; T. Scown, DuPont Crop Protection; S. Loutseti, Du Pont Hellas S.A.; R. McKelvey, DuPont Haskell Global Centers for Health and Environmental Sciences; J.W. Green, DuPont / Applied Statistics Group. Registration of sulfonylurea (SU) herbicides is complicated by the fact that *Lemna* species, the model aquatic macrophyte, are highly sensitive to these compounds. Higher tier lines of evidence are therefore required in order to demonstrate low risk to aquatic macrophytes. However, there are differences and significant uncertainties in the higher tier approaches that individual regulatory bodies find acceptable. Our general strategy for demonstrating low risk for SUs, described here with data for 5 SUs, relies on multiple and independent lines of evidence including: 1. Use of toxicity data from testing on additional aquatic macrophytes to calculate the geometric mean and the HC5 and lowering of the uncertainty factor, since data from testing on additional aquatic macrophytes show other species can be orders of magnitude less sensitive than *Lemna*. 2. Use of data from exposure studies with SUs to *Lemna* under cold conditions to simulate early spring or late fall applications. During these periods where the plants are not actively growing, toxicity is greatly reduced. 3. Use of data from variable-length exposure studies to address exposure scenarios with short exposure durations. Standardized toxicity tests on *Lemna* rely on keeping SU water concentrations constant over the 14- or 7-d exposure period, however concentration profiles produced by exposure modeling often shows peaks of very short duration. Use of data from multiple testing strategies allows for alleviation of uncertainties associated with any approach in isolation and also allows for a weight of evidence approach to the risk assessment.

**RP043 Extrapolation across plant species and endpoints in tiered risk assessment frameworks: are lower tier species and endpoints always protective?** G.H. Arts, Alterra Wageningen University and Research Centre / Centre for Water and Climate, Environmental Risk Assessment, Alterra, part of Wageningen University and Research Centre / Environmental Risk Assessment; T. Brock, Wageningen UR; E. Dorsman, Alterra, part of Wageningen University and Research Centre; A. Jonas, Alterra, part of Wageningen University and Research Centre; E. Smit, RIVM, National Institute for Public Health and the Environment. Ecological effect assessment for pesticides typically relies on the testing of standard test species in the 1st Tier of the risk assessment. Other species and endpoints, different from the standard ones, are protected by the application of assessment factors. In the EU, pesticide effect assessment follows a tiered approach. Ideally, higher tiers are more realistic and less conservative than higher tiers. For a few, data-rich herbicides, enough data are available to test the protectiveness of the lower tiers for the higher tiers in current regulatory frameworks. The available data indicate that the Tier 1 effect values and Regulatory Acceptable Concentrations are not always protective for Hazard Concentrations deduced from a range of species and endpoints as assessed by means of the Species Sensitivity Distribution (SSD) method. This is especially the case when Tier 1 is not based upon the most sensitive species, e.g. when a sediment-rooted macrophyte is more sensitive than the standard floating test species *Lemna* spp. This is the case for specific Modes of Action. If in that case the effect value for the sediment-rooted *Myriophyllum spicatum* is considered with a safety factor, this value is protective for effects on other submerged macrophytes. Also in the case of photosynthesis inhibitors, while plants exhibit a comparable range in sensitivity based on inhibition of photosynthesis, the standard Tier 1 species are not always protective for other species and endpoints. In general, hazardous concentrations from species distributions were below



population and community NOEC values from mesocosm studies, although differences were sometimes small. Laboratory toxicity tests with rooted macrophytes, as well as SSDs based on these toxicity data, are sensitive tools in the risk assessment.

**RP044 Potential bioaccumulation of endocrine disrupting chemicals in leafy vegetables** J. Lu, J. Wu, P.J. Stoffella, University of Florida/IFAS Indian River Research and Education Center; P. Wilson, University of Florida / IFAS / IRREC- Soil & Water Science. Lettuce plants were grown hydroponically in solutions spiked with common endocrine disrupting chemicals in order to evaluate their potential uptake and accumulation in edible tissues. In addition, plants were also grown in non-spiked media and the target EDCs were applied to the foliage in water simulating overhead irrigation with reclaimed water. An isotope dilution method was developed to evaluate the potential bioaccumulation of the EDCs, including nonylphenol (NP), bisphenol (BPA),  $\beta$ -estradiol (E2), and estrone (E1). Isotope labeled standards of each compound, which were used as the internal standard (IS), and the surrogate  $\beta$ -estradiol 17-acetate were added at the beginning of the extraction. The matrix was homogenized and extracted ultrasonically with acetone. The extract was then subjected to non-specific acid hydrolysis and trimethylsilyl derivatization. The derivatized samples were analyzed by gas chromatography-tandem mass spectrometry (GC-MS/MS). The hydroponic studies with lettuce demonstrated that NP, BPA, and E2 could all accumulate in lettuce. The concentrations of NP, BPA, and E2 in leaves from the root exposure treatments were 137.08, 124.97, 176.09  $\mu\text{g kg}^{-1}$ , respectively. Uptake was more efficient for foliar exposure treatment for all of the EDCs except for E1. The concentrations of NP, BPA, and E2 in leaves from the foliar exposure treatments were 176.17, 167.76, and 209.94  $\mu\text{g kg}^{-1}$ . The bio-translocation factors (TF) from root to edible leaves for NP, BPA, and E2 in the root exposure treatments were 0.039, 0.520, and 0.863, respectively. The TF from leaf to root in foliar exposure treatments for NP, BPA, and E2 were 0.335, 0.636, and 0.281, respectively. The bioaccumulation of E1 was different from the other EDCs. E1 did not accumulate in the plants, but E2 did, suggesting that E1 is transformed into E2 in the plant. E2 concentrations in E1 exposure treatments (including foliar and root exposures) ranged from 38.62 to 99.44  $\mu\text{g kg}^{-1}$ .

**RP045 Uptake and Accumulation of Four PPCP/EDCs by Two Edible Vegetable Species** L. Dodgen, University of California Riverside / Department of Environmental Science; J. Gan, University of California Riverside. Water scarcity has prompted increased use of reclaimed water for irrigation, but many pharmaceutical and personal care products (PPCPs) and endocrine-disrupting chemicals (EDCs) are routinely detected in reclaimed water. The intersection of these two situations creates concern about human health risks from exposure to PPCPs/EDCs in food. To examine potential for plant uptake and accumulation of PPCP/EDCs during irrigation with reclaimed water, Batavia 'Nevada' lettuce (*Lactuca sativa*) and collards (*Brassica oleracea*) plants were grown hydroponically in growth chambers for 21 d with nutrient solution that was spiked with  $^{14}\text{C}$ -labeled bisphenol A (BPA), diclofenac sodium (DCL), naproxen (NPX), or nonylphenol-111 (NP). Spiked concentrations were 46.4, 237.4, 178.2, and 110.4 ng/L, respectively, which are representative of these chemicals' levels in reclaimed water. Nutrient solution was renewed every 3 d. Plant treatments had significantly greater removal of  $^{14}\text{C}$ -chemical from the hydroponic solution for BPA, DCL, and NPX as compared to treatments without plants. Treatments with NP showed high rates of volatilization, resulting in no significant difference in nutrient solution concentrations among different treatments. Plant accumulation followed the decreasing order BPA > NP > DCL > NPX across compounds and roots >> new leaves > old leaves > stem across plant tissues, with concentrations ranging from  $0.22 \pm 0.03$  ng/g for BPA in lettuce new leaves to  $926.89 \pm 212.80$  ng/g for BPA lettuce roots. Anionic chemicals (DCL and NPX) had significantly less accumulation than neutral chemicals (BPA and NP) for collards, but no significant difference was observed between lettuce treatments. Plant structure and species, as well as chemical charge, appeared to strongly affect accumulation of the tested PPCP/EDCs into plants.

**RP046 Effects of humic substances on adsorption of dieldrin in soil** H. Murano, Meijo University / Faculty of Agriculture; M. Takechi, K. Kakishita, T. Isoi, Meijo University; S. Nobuyasu, T. Otani, National Institute for Agro-Environmental Sciences, Japan. Dieldrin is one of persistent organic pollutants (POPs) and organochlorine pesticides. Although the use

of dieldrin has been restricted or banned for more than three decades, the chemical is still detected in some crops, especially in cucurbits. Organic chemicals with high hydrophobicity tightly adsorb on soil particles, and thus usually these chemicals are hardly taken up by plants from soil. Furthermore, the residual dieldrin in soil must have changed to bound residue, and most of it cannot be taken up by plants. However, some POPs including dieldrin, which have existed over three decades, are somehow taken up by cucurbits, which suggests that bioavailable POPs still exist in those soils. In the present study, the existence form of dieldrin in soil was examined. It has been suggested that the main adsorption sites of POPs on soil are soil organic matters. Therefore, both humic and fulvic acids and humin were extracted from soil, and the adsorptions of dieldrin on these humic substances were examined by batch method. Dieldrin in soil without any treatment reached the adsorption equilibrium after 24 hours, and the amounts of adsorption of dieldrin did not change for 24 to 96 hours. The dieldrin adsorbed on the soil and had a high  $K_d$  value of 50,000 ( $\text{kg L}^{-1}$ ). The adsorption amounts of dieldrin on soil removed humic and fulvic acids were lower than those on the soil without treatment. These results suggest that humic acid and fulvic acids affected on the adsorption of dieldrin on soil.

**RP047 Analysis of triclosan uptake in plant tissue** R.S. Prosser, University of Guelph / School of Environmental Sciences; L. Lissmore, University of Guelph / Laboratory Services; P. Sibley, University of Guelph / School of Environmental Sciences. Triclosan is an antimicrobial chemical that is present in a variety of personal care products. Due to the relatively high persistence and hydrophobicity of triclosan, the chemical partitions in the solid portion of municipal sewage. Triclosan continues to persist after the solid sewage is processed into biosolids. There is concern about whether triclosan is taken up in roots and translocated throughout plants that are grown in biosolids-amended soil. In order to answer this question, a modified analytical method was developed to analyze triclosan in plant tissue. Soxhlet extraction was used to extract triclosan from the plant tissue, followed by solid phase extraction with hydrophilic-lipophilic balance columns. Triclosan was quantified using LC-MS-MS. A labeled triclosan internal standard was used to account for matrix effects. Soybean (*G. max*), radish (*R. sativus*), and carrot (*D. carota*) were then grown in soil amended with anaerobically dewatered biosolids. Biosolids were spiked with varying quantities of triclosan to produce a range of environmentally relevant exposures. Plants were grown under environmentally controlled conditions and harvested midway through their life cycle and at maturity. Root, stem, and edible portion of the plant were analyzed using the modified analytical method. Triclosan was present in plants that were grown in biosolids-amended soil but in very low concentrations. In all three species, there was no difference in concentration of triclosan among portions of the plants within treatment, nor among treatments within portion of the plant.

**RP048 Phytoremediation of Atrazine from Simulated Surface Water Runoff using Switchgrass** V.C. Albright, I. Murphy, Iowa State University / Entomology; J. Anderson, Iowa State University; J.R. Coats, Iowa State University / Entomology. Atrazine is one of the most widely used herbicides in the agricultural industry today and, as a result, can commonly be found in many bodies of water in the Midwest. Atrazine and its metabolites have been shown to have many adverse effects on plants and animals within an ecosystem. Previous research has shown that switchgrass can take up and degrade atrazine into its less toxic metabolites. The current research project has two objectives: (1) determine degradation of atrazine using radiolabeled  $^{14}\text{C}$ -atrazine to allow for more sensitivity than the previous studies which used gas chromatography and (2) determine the possible exudation of atrazine metabolites from the switchgrass after uptake and degradation. The research presented here shows that atrazine was taken up and degraded into the metabolites deethylatrazine, deisopropylatrazine, didethylatrazine, and cyanuric acid. Contrary to published research, we did not find the metabolite hydroxyatrazine. Additionally, the concentration of atrazine and its metabolites within the switchgrass tissues is below the EPA's 4 ppm tolerance for range grasses. Thus, the transfer of atrazine and its metabolites through the food web should be limited. Finally, we showed that switchgrass did not exude metabolites of atrazine into soil following uptake and degradation.

**RP049 Environmental Assessment and Remediation at Clinton Creek Asbestos Mine: Can Wild Barley be Used for Stabilization?** M. Dodd, Royal Roads University / School of Environment & Sustainability, Royal Roads University / School of Environment & Sustainability; B. Dushenko,

Yukon College. Ecological footprints have resulted from former mining activities at the abandoned Clinton Creek Asbestos Mine, located approximately 100 km northwest of Dawson City in the Yukon Territory. These include large expanses of virtually barren and unstable areas of tailings and waste rock. Geochemical and quantitative environmental assessments of the terrestrial habitats were conducted using soil, water, sediment and vegetation (wild barley – *Hordeum jubatum*) sampling and analysis. Ground cover vegetation surveys were conducted in representative areas directly and indirectly affected by the mine tailings or waste rock. Metal bioaccessibility was also determined using physiologically based extraction tests. Concentrations of As, Ba, Cr and Ni were elevated in sediment, and soil samples; however, these geologically elevated levels were not accompanied by elevated concentrations in water samples in relation to Canadian environmental quality guidelines. The high pH of soil samples has likely kept the metals out of solution and prevented their movement into surrounding waters. Metal bioaccessibility values were also relatively low. Chronic risks from the abandoned mine owing to geochemical mobilization of metals/metalloids or other substances to the water appeared to be unlikely. Elevated As, Cr and Ni concentrations were detected in the wild barley samples compared to control samples; however metal enrichment levels were less than unity. Given that waste rock and tailings deposits at the site are unstable and continue to slump and redistribute, and contaminant enrichment levels in plants is low, it is hypothesized that wild barley and other vegetation could help to naturally stabilize these areas.

**RP050 Evaluating the potential impact of deposition from static rocket testing on crop plants** W.J. Doucette, S. Mendenhall, L. McNeill, Utah State University / Utah Water Research Laboratory. Tests of horizontally restrained rocket motors at the ATK facility in Promontory UT, USA result in the deposition of an estimated 1.5 million kg of entrained soil and combustion products (largely aluminum oxide, gaseous hydrogen chloride and water) over areas as large as 80-130 km<sup>2</sup> and at distances up to 16-20 km from the test site. As the cloud cools, the deposition material, referred to as Test Fire Soil (TFS), drops on the surrounding area. Local residents and farmers observing TFS deposited on their gardens, fields, and crops have expressed concerns regarding the potential impact of this material. To address these concerns, we conducted greenhouse studies with corn and alfalfa exposed to TFS, through various soil and foliar applications. The impact of the TFS was evaluated by comparing the growth and tissue composition (i.e. nutrient content, metals, anions) of controls relative to the treatments. Exposure to TFS, containing elevated levels of chloride, aluminum and pH (likely due to the high temperature conversion of calcium carbonates to calcium oxide) relative to native soils, affected the germination, growth, and tissue concentrations of various elements, depending on the type and level of exposure. Germination was inhibited by high concentrations of TFS in soil but the impact was minimized if the TFS was pre-leached with water. Corn and alfalfa biomass production was reduced in the 10% TFS/soil mixture relative to controls and corn grown in soils treated with TFS did not develop kernels. Tissue concentration differences between controls and TFS exposed plants were observed for some elements. Foliar applications of TFS had no significant impact on biomass production but did show some difference in the elemental composition of leaves relative to controls. Washing the TFS off the leaves after 7 days lessened the impact. Results to date indicate that the TFS deposition could have an effect depending on the amount and growth stage of the crops but the impact could be mitigated with application of additional irrigation water. The high level of chloride associated with the TFS is likely the main cause of the observed impacts.

**RP051 Nonpolar organic contaminant partitioning in Norwegian rivers** I.J. Allan, Norwegian Institute for Water Research, Norsk Institutt for Vannforskning; S.B. Rannekleiv, Norwegian Institute for Water Research; C. Harman, Norwegian Institute for Water Research (NIVA). Riverine chemical contamination is complex due to the multitude of possible different contaminants, their varying natural/anthropogenic sources and their bioavailability being dependent on many factors. A variety of pathways including point sources from wastewater treatment and industry, run-off, long and short range diffuse sources and re-introduction from within system sinks such as river bank or bottom sediments. Well-studied HOCs such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and chlorinated pesticides (OCs), or relatively newer brominated flame retardants (e.g. brominated diphenylethers, PBDEs) may be present in surface waters at low yet toxicologically relevant concentrations. Sorption

and partitioning of these contaminants occur onto surfaces, colloids and into organic matter present in the particulate and dissolved phases. This in turn affects their bioavailability and mobility and can result in extremely low dissolved concentrations in water. Exceptionally strong HOC sorption to soot-like organic phases in sediments has been observed in recent years. Most of this work however has focussed on bottom sediments and little is known of the partitioning in the overlying water phase. The fraction of contaminants present in surface waters sorbed to particulate organic matter and available for partitioning into the dissolved phase is not often well-characterised. This work aimed to investigate the partitioning of HOCs between suspended particulate matter and the dissolved phase in rivers of contrasting properties. This work took place between 2008 and 2010 in rivers representative of large catchments with relatively low level of urbanisation and smaller urban streams. A combination of passive sampling with low density polyethylene, silicone or semipermeable membrane devices and suspended particulate matter sampling (time-integrative sampling/continuous flow centrifuge) was applied to six Norwegian rivers. Passive sampling data allow us to establish baseline concentrations for PAHs, PCBs/OCs and PBDEs as well as the magnitude of increases observed for urban rivers. Organic carbon-normalised partition coefficients for PAHs were high and higher than those expected from empirical relationships. The degree of inter/intra river variability was high indicating that more work is necessary to understand it. Overall riverine fluxes of these HOCs to coastal areas are discussed.

**RP052 Reach for the Peak: Persistent Organic Pollutants in Headwater Catchments of Ireland** H.E. Scott, Trent University / Environmental & Life Sciences, Trent University / Environmental and Life Sciences; J. Aherne, Trent University; C.D. Metcalfe, Trent University / Environmental & Resource Studies, Trent University / Department of Environmental & Resource Studies; T. Metcalfe, Trent University / ERS. Long-range atmospheric transport of persistent organic pollutants (POPs) is a global concern as evidenced by the enactment of the Stockholm Convention on Persistent Organic Pollutants in 2001. Ireland is located on the western periphery of Europe and is assumed to receive 'clean' Atlantic air. As such, it has been used as a baseline for other regions to compare against (e.g., continental Europe). Nonetheless, few studies have evaluated background concentrations or biogeochemical interactions of POPs within the Irish environment. In the current study, POPs were measured at five upland lake catchments in oceanic regions around Ireland. Surface soils were collected during October 2010 and Semi-Permeable Membrane Devices (SPMDs) were deployed for a 6-month period in the lakes. The lake catchments were chosen in high elevation regions (500 – 800 meters above sea level) to avoid influences of local emissions and are believed to be 'pristine' sites. In addition, the source-regions of atmospheric inputs to the catchments were evaluated using back-trajectory analysis, which estimated the incoming proportions of air at the sites for 1, 2, 3, and 5 five day intervals. As anticipated, the concentrations of POPs were relatively low at each of the study sites given the remote nature of the catchments. Nonetheless, polychlorinated biphenyls (PCBs), several classes of organochlorine pesticides (OCPs), and polycyclic aromatic hydrocarbons (PAHs) were detected in some or all of the lakes and soils. Notably, polybrominated diphenyl ethers (PBDEs) were identified in soils at a number of the study areas; however, PBDEs were below detection limit for SPMD analysis in this study. The compounds partitioned between lake and soil compartments based on their volatility and hydrophobicity; for example, more volatile substances such as HCHs and mono to penta-chlorinated biphenyls were found in higher proportions in lakes, whereas, PAHs and hexa to deca-chlorinated biphenyls were more prominent in soils. Concentrations of Endosulfan and PAHs were highest at the east coast sites potentially due to the influence of national sources or contributions from European airsheds. In contrast, PCBs exhibited a negative correlation with easting and many of the congeners (penta, hepta, octa, and nona-chlorinated groups) illustrated a tentative relationship with annual rainfall and soil organic matter.

**RP053 Species-Specific Uptake of Dissolved Polychlorinated Biphenyls (PCBs) in Streams** V.D. Dang, University of Florida / Physiological Sciences; C.M. Lee, Clemson University / Department of Environmental Engineering and Earth Sciences; D.M. Walters, US Geological Survey. Leaf detritus is an important source of organic matter in lotic systems. These leaves are covered with biofilm that sorbs persistent organic pollutants (POPs) from the surrounding water, and further serve as a vector of contaminants to food webs. Many studies have focused on rates of leaf breakdown among different species, but none has assessed the effect of leaf quality (e.g.,

C:N and lignin content) on uptake rate of contaminants. In this research, we used leaf species of red maples (*Acer rubrum*), northern red oak (*Quercus rubra* L.), tulip poplar (*Liriodendron tulipifera*), and evergreen rhododendron (*Rhododendron maximum*) to determine uptake rates of PCBs. These leaf species differ in their initial chemical composition, and therefore are subject to differential microbial colonization that drives decomposition and uptake rates. We prepared single- and mixed-leaf bags and deployed them in the streambed of Town Creek/Lake Hartwell Superfund site, SC, for 10, 20, 30, 50, 70, and 90 days. Analytical tools including HPLC and GC/ECD were used to measure content of ergosterol (fungal biomass) and PCB uptake, respectively. Fungal biomass serves as an important indicator of microbial colonization, which provides insight into the uptake of PCBs in leaves. In single-leaf bags, we expect that leaves with higher nutritional quality (e.g., maple, oak, and tulip poplar) accumulate PCBs to a greater uptake rates than lower quality leaves (e.g., rhododendron) due to faster microbial conditioning and rather than higher ergosterol concentrations. Conversely, patterns of PCB uptake are likely more variable in the mixed-leaf bags than in the single-leaf bags. Quantifying species-specific differences in PCB uptake among leaves that compose the detritus pool in streams is an important step to improve our understanding of entry points of POPs into stream food webs.

**RP054 The fate of high volume personal care product chemicals (fatty alcohols) in riverine environments: the effect of treatment type and Eco-region** S.M. Mudge, Exponent; P.C. DeLeo, American Cleaning Institute / Environmental Safety, American Cleaning Institute. Fatty alcohols are one of the major surfactant classes used in personal care products. Previous studies into the source and fate of fatty alcohols passing through wastewater treatment plants (WWTPs) clearly demonstrated that the compounds in the influent of WWTPs were removed primarily with the solids or were degraded during the secondary, biological, stage. The fatty alcohols present in the effluents were derived from new bacterial synthesis in the works. Some doubt might have existed regarding the general applicability of these findings since these studies were both conducted using activated sludge stages. A new study was undertaken at 24 different WWTPs in three different eco-regions of the USA. Six different secondary treatment stages were considered including lagoons which were thought to be less efficacious in compound removal. Studies were conducted in Oklahoma, Ohio and Oregon with eight WWTPs sampled in each. Along with the fatty alcohol chain length profiles being quantified, the stable isotopic composition of each fatty alcohol was measured. The  $d^{13}C$  and  $d^2H$  values were measured for each compound. Data from commercially available products was previously collected from a catchment in Virginia and also projected into the cross plots. Several different sources of fatty alcohols could be identified; terrestrial plant compounds with  $d^{13}C$  values around -34‰ were clearly separated from both petrochemical-derived and oleochemical-derived compounds. Influent samples had fatty alcohols consistent with a mixture of faecal matter and detergent products. The effluents had significantly different signatures that were not associated with the either the detergents or the faecal lipids. The sediments from the rivers downstream of the discharges had sterols / stanols present indicating discharges from the WWTPs although the fatty alcohols were predominantly from terrestrial plants and secondly from *in situ* algal synthesis. The influents to the WWTPs were consistent within each eco-region but significantly differed between eco-regions. This may be due to differences in the product preferences between regions. Multivariate statistical analyses confirmed that there were differences in the fatty alcohol profiles between the sample types and between eco-regions, but no differences were detected between WWTPs. The mean removal from the influent was >97% although the effluent fatty alcohols had a different stable isotopic signature to those of the influent.

**RP055 Fate and Transport Modeling of PCBS in the Houston Ship Channel Estuary** N.L. Howell, H.S. Rifai, University of Houston / Civil & Environmental Engineering. The Houston Ship Channel (HSC) is a highly industrialized corridor both for shipping and petrochemical activities. As is the case with many such industrialized areas, there are high concentrations of hydrophobic organic pollutants (HOPs) such as polychlorinated biphenyls (PCBs). This study brings together PCB data from five separate sampling events between 2002 and 2009 in several media to develop a hydrodynamic water quality model using Environmental Fluid Dynamics Code (EFDC). The model incorporates specific known PCB sources from industry, urban runoff, and Superfund sediment sites to quantify external

loads and internal PCB contaminant transport. There were two specific research objectives in view during the model's development. The first was to understand the role of the bed sediment with regard to contaminant distribution and sourcing. It is fairly clear that the bed sediment represents a large repository of PCBs extending throughout the HSC, with some areas that are highly localized while others span nearly an entire reach of the estuary. However, due to complex pathways for the movement of bed sediment and the bioavailability of PCBs in the bed sediment, it is not as clear how large or small a role bed sediment play with regards to overall PCB loading and risk as compared with other sources. The second research objective was to evaluate temporal trends in water and sediment PCB contamination in the HSC, especially with regard to the most upstream and most highly industrialized area of the HSC. Monitoring efforts showed that PCB contamination generally seemed to be decreasing from 2002-2008, and yet concentrations increased from 2008-2009. Unusually long dry periods and Hurricane Ike are possible explanations for the increase. Our results indicate that a significant portion of the PCBs in bed sediments is not bioavailable due to the levels and type of organic carbon present in them (effectively reducing the bed sediment PCB risk to the aquatic food web) and that there are highly contaminated bed sediments in the upper reaches of the HSC that are not transported further downstream due to lower water velocities. These highly contaminated areas will only be mitigated through sediment burial and natural attenuation processes such as biodegradation.

**RP056 Nothing to declare? Decision thresholds associated with multiple measurements, information reporting and their application** G. Manificat, IRSN-Institute for radioprotection and nuclear safety / Environmental radioactivity study and monitoring department. Testing for radionuclides or chemicals in environmental matrices often leads to ask questions about their presence and the way to report the measurements. Detection limits, reporting limits, limits of quantitation, critical levels, and lowest limits of detection are various names and concepts for reporting results and the degree of confidence put in them. In fact, when sample activity (concentration) is measured several times for various reasons, then with each measurement can be associated an individual decision threshold and limit of detection. Each measurement can be analyzed through its own decision threshold. Reporting a measurement only as lower than a limit (reporting, detection or otherwise) is a loss of information. Furthermore, the whole measurements can sometimes present contradictory results, with measurements being lower than the decision threshold and others higher. How can you build a decision threshold and a detection limit taking into account all the individual results? Can you decide if the radioactivity is finally detected or not with a given degree of confidence and how do you report that? It is interesting to note that the global decision threshold, taking into account all individual results, could enable the analyst to decide that the radioactivity is present even with each individual result negative in terms of individual decision threshold. We are able to show how these thresholds and these coherent limits cumulated can be determined. In a general way a rigorous method of cumulating makes it possible to systematically decrease the decision threshold and limit of detection in terms of activity. This approach, which tries to exploit all the information in the measurements, has interesting applications in periodical environmental measurements. It also implies the reporting of some information which, if one apply the standard reporting procedures would be lost or erased. This reporting is simple and can be compatible with any kind of limits chosen (detection, reporting, decision or otherwise). Examples from measurements realized by IRSN within the framework of the national monitoring of the environment will illustrate the potential impact of these methods on the final assessments.

**RP057 A Different Approach to Detection Limits** E. Rodgers, R. Forman, R. Vitale, Environmental Standards, Inc.. State Agencies often require data to be reported to the method detection limit (MDL) for various programs, typically for the purpose of meeting health-based standards or for demonstrating compliance with a discharge permit. Commercial environmental laboratories often perform MDL studies in accordance with 40 CFR Part 136, Appendix B to determine matrix-specific and analyte-specific MDLs for a given preparative technique and analytical method. This method of determination of the MDL has endured significant technical criticism but remains the most wide-spread method for determining the MDL. Unfortunately, calculating the MDL at the 99% confidence interval allows for the probability that 1% of the samples analyzed with a true concentration at the MDL will be reported as false negatives. Additionally, reporting



data to the MDL does not control the possibility for false positives given the propensity for a Gaussian distribution of environmental sample results about the mean. In order to generate detection limits with a high degree of defensibility and show that a particular analyte can be "seen" at the MDL, project-specific MDLs for metals analyzed by ICP and ICP/MS in aqueous, solid, and biological matrices were developed for a large multi-year environmental cleanup utilizing months of blank data. Additionally, with each analytical sequence over years of metals analyses, a series of MDL standards were required to be analyzed to demonstrate the laboratory's ability to detect the project-specific MDL. This paper will address the development of project-specific MDLs and the results from implementation of these MDLs; several years of data for the MDL standards will be presented.

**RP058 Comparison of detection procedures applied to steroid hormone GC/MS/MS and pharmaceutical LC/MS/MS methods** B.T. Foreman, R.C. ReVello, U.S. Geological Survey / National Water Quality Laboratory; E.T. Furlong, M.C. Noriega, J.L. Gray, V.K. Jha, U.S. Geological Survey. Procedures for estimating contaminant detection levels often are prescribed based on regulatory requirements or chosen based on actual or perceived data user need or presumed simplicity. For example, the U.S. Environmental Protection Agency's method detection limit (USEPA MDL) procedure often is mandated when USEPA methods are used or for studies conducted for federal, state, or local regulatory agencies. The MDL procedure has several well documented limitations, one being the requirement that a cumbersome iterative spiking approach be used to ensure that the analyte concentration in spiked test samples is within 1–5 times the determined MDL. This approach is impractical, in part, because it is difficult and expensive to create a solution of analytes, each at the appropriate concentration, for methods with many analytes having differing instrument-response characteristics. A comparison of procedures for estimating detection or reporting levels was undertaken for two water methods recently developed by the USGS National Water Quality Laboratory: a solid-phase extraction, compound derivatization GC/MS/MS method for steroid hormones, and a direct aqueous injection LC/MS/MS method for 112 pharmaceuticals. Detection procedures compared included the USEPA's MDL and lowest concentration minimum reporting level (LCMRL) procedures, and ASTM International's interlaboratory detection estimate (IDE; applied intralab) procedure. The IDE and LCMRL procedures are based on a multi-concentration (calibration-like) spiking approach, and the calculators developed for these procedures also estimate the MDL and Currie's critical level (Lc), which in theory should be nearly equivalent to the MDL value. Indeed, estimated Lc and MDL concentrations were nearly equivalent to each other for some hormone and pharmaceutical methods' analytes, demonstrating the operational advantage of using either multi-concentration spiking procedure compared to the iterative MDL procedure. For some analytes, the estimated detection levels, which are based on the response of the quantitation ion, were substantially lower than the "true" detection level because the responses of secondary confirmation ions were too low. For several blank-limited analytes, detection levels also were estimated using lab blank data that provide a more accurate, direct estimate compared to detection level estimates from spike-based (MDL-type) approaches, which often are artificially low.

**RP060 Calibration weighting for low-level environmental data: An example from the analysis of estrone** J.L. Gray, U.S. Geological Survey, USGS / National Water Quality Laboratory; B.T. Foreman, R.C. ReVello, U.S. Geological Survey / National Water Quality Laboratory. In order to accurately convey quantitative environmental chemistry results, it is useful to have some estimate of measurement uncertainty. Overall uncertainty can result from uncertainty in the fit of calibration data (calibration error) and from other non-calibration uncertainties. Calibration data typically are fitted using a least-squares model, which may or may not be weighted to minimize the effect of the highest calibration levels on the fitted equation. A gas chromatographic-tandem mass spectrometric technique was used to measure the estrogenic hormone estrone, having typical environmental sample concentrations of 10 ng/L or less. Three calibration models were compared over a range of 1 to 1,000 pg/mL (equivalent to approximately 0.4 to 400 ng/L estrone in water samples), and all three models had less than 5% error at the high end (1,000 pg/mL) of the calibration range. However, at 10 pg/mL (4 ng/L equivalence for a water sample), the unweighted model displayed an error of nearly 100%. Applying a  $1/x$  (where  $x$  is the standard concentration) weighting factor for the regression model reduced this error to less than 10%, while a weighting factor of  $1/x^2$  reduced the error to less

than 2%. The unweighted model is the best representation of the data greater than 160 ng/L, the  $1/x$  weighting is the best representation from 12 to 160 ng/L, and the vast majority of the environmental detections (less than 12 ng/L) are best represented by the  $1/x^2$  weighting. At typical ( $\leq 10$  ng/L) environmental concentrations of estrone, uncertainty derived from an unweighted calibration curve far exceeds all other sources of method uncertainties. Thus, uncertainty from calibration can be virtually eliminated by application of a  $1/x^2$  weighting model for most environmental samples containing estrone.

**RP061 Differences in the proteome of seagrasses from the Mississippi-Alabama coast during and after the Deepwater Horizon oil spill** J. Corrales, University of Mississippi / Department of Pharmacology; K.L. Willett, University of Mississippi / Environmental Toxicology Research Program; M. Dailey, University of Mississippi / Department of Pharmacology and Environmental Toxicology Research Program. The conservation of seagrass beds is important both from an environmental and economic standpoint. Seagrasses provide at least 13 ecological roles some of which are provision of grazing and nursery grounds, support of detrital food webs, wave protection and sediment stabilization. Their economic value is clear as 70–90% of commercial fish rely on seagrass habitat for part of their cycle. Also, it is estimated that one seagrass acre is worth \$9,000 to 28,000. In the northern Gulf of Mexico, seagrass coverage has been estimated to be 2.5 million acres [Environ Pollut (2007) 146:206–218]. However, seagrass beds are in decline worldwide due to environmental factors (changes in temperature, salinity, erosion, etc.) and anthropogenic factors (most importantly toxic chemicals). Anthropogenic impacts such as the Deepwater Horizon oil spill (DWHOS; the largest marine oil spill in the history of the petroleum industry with over 200 million gallons of crude oil spilled into the northern Gulf of Mexico) put at risk seagrasses and, in turn, all other species that rely on them for survival. At a molecular level, adaptability of organisms to an environmental or anthropogenic insult can be monitored by changes in protein expression (biological function). Currently though, little is known about the constitutive and inducible expression of seagrass proteins. In the present study, changes in the seagrass proteome during and after the DWHOS were documented providing a baseline database of species, spatial and temporal variation. Three species of seagrasses (*Halodule wrightii*, *Ruppia maritima* and *Thalassia testudinum*) and associated sediment samples for analysis of DWHOS contaminants were collected at various sites from May to October 2010 along the Mississippi-Alabama coast. The presence of polycyclic aromatic hydrocarbons, oil spill contaminants, in sediments was measured by gas chromatography mass spectrometry (GC/MS). Proteomic differential expression in the three species of seagrasses was determined using a bottom-up proteomics approach where proteins were digested into peptides and analyzed by liquid chromatography coupled with MS. Differentially expressed proteins were identified by the Protein Lynx Global Server software. Heat shock proteins and 14-3-3 proteins were consistently expressed. Other stress response proteins (SOD, catalase, etc.) were also found. Supported by Northern Gulf Institute 191001-306811-02 / TO 002 and NIUST NA07OAR4300494.

**RP062 Interactions between climate change, contaminants, and ecosystems in the North Atlantic Landscape Conservation Cooperative** A.E. Pinkney, U.S. Fish & Wildlife Service / Chesapeake Bay Field Office; D.A. Cristol, College of William and Mary / Biology department; C.T. Driscoll, Syracuse University / Department of Civil and Environmental Engineering; D.C. Evers, Biodiversity Research Institute; M.J. Hooper, U.S. Geological Survey / Columbia Environmental Research Center; J.W. Jones, U.S. Fish and Wildlife Service / Virginia Field Office; R.S. Lazarus, U.S. Geological Survey / Patuxent Wildlife Research Center; A. Milliken, U.S. Fish and Wildlife Service / Northeast Regional Office; B.A. Rattner, U.S. Geological Survey / Patuxent Wildlife Research Center; J. Schmerfeld, U.S. Fish and Wildlife Service / Division of Refuges; D.W. Sparling, Southern Illinois University / Cooperative Wildlife Research Center; T.H. Tear, The Nature Conservancy. In July 2011, SETAC sponsored an international Pellston workshop entitled, "The Influence of Global Climate Change on the Foundations of Environmental Toxicology and Chemistry." The goal was to increase understanding on how global climate change may affect the environmental fate, bioavailability and toxicity of chemicals. Papers from the work groups are forthcoming in *Environmental Toxicology and Chemistry*. Using the workshop's framework, we are examining the interactions between climate change and contaminants in the North Atlantic Landscape

Conservation Cooperative (NA LCC), a focus area for research and prioritization of conservation issues comprising much of the Northeast U.S. and the Maritime Provinces of Canada, home to over 50 million people. Our goal is to evaluate the state of the literature and communicate the findings in order to prioritize research needs and inform land managers. We obtained and summarized the results of climate reports within the NA LCC in terms of documented and projected changes. The key parameters include summer and winter temperature; quantity, intensity, and patterns in precipitation; timing of migrations, breeding, and bloom; losses in the extent and duration of ice cover; and rising sea level. We divided the region by broad habitat category (terrestrial, freshwater, and estuarine/marine) with wetlands distributed according to these categories. We gathered contaminant data within sub-regions of the NA LCC and evaluated data on patterns and trends in contaminant releases and environmental concentrations and the predicted shifts in agriculture and pesticide use. Here, we present case studies detailing the documented and predicted interactions for specific chemicals of concern within geographic areas such as Adirondack Mountains, coastal wetlands, and Chesapeake Bay tributaries. One example that crosses many habitats is mercury, which is responsible for many of the region's fish consumption advisories and is also a concern for multiple species of wildlife in terrestrial and aquatic habitats. We describe projected changes in methylmercury bioavailability and effects in the NA LCC due to altered precipitation patterns, hydrology and temperature along with forecasted changes in global, regional, and local emissions. Finally, we discuss the limitations and uncertainties in the analysis and prioritize research needs to allow greater understanding and prediction of climate change interactions with chemicals in NA LCC ecosystems.

**RP063 Assessing the Risk of Municipal Wastewater Treatment Plant Discharges in the Context of Multiple Stressors** K. Kapo, Montani Run, LLC; S.D. Dyer, The Procter & Gamble Company / Central Product Safety, The Procter & Gamble Company / Miami Valley Innovation Center, The Procter & Gamble Company / Miami Valley Labs. It remains a challenge to accurately assess the ecological risk of municipal effluent discharges in the larger context of multiple stressors. Potential biological impacts associated with domestic "down-the-drain" chemicals were previously evaluated by statistical comparisons of upstream and downstream fish and invertebrate communities from municipal wastewater treatment facility discharges points for data from the time period 2000-2007 (Ohio EPA data). Results of this study compared to an earlier analysis of 1996-1999 data indicated improvements in both water quality and receiving biota downstream of discharges, particularly in high-population density areas. This current study further explores temporal changes in treatment efficiency by examining historic violation patterns between the 1996-1999 and 2000-2007 studies using U.S. EPA's Permit Compliance System (PCS). The influence of treatment efficiency on the detection of differences in the condition of upstream and downstream biological communities was directly evaluated by a re-analysis of the 2000-2007 data in which facilities were categorized by violation frequency. In addition, in order to more specifically assess the influence of effluent on biological communities in the context of multiple stressors, a variety of water chemistry factors often associated with effluent (such as BOD, ammonia, etc.) and corresponding biological community condition were assessed at monitoring locations with varying levels of effluent exposure. The results of this analysis underscore the additional significance of non-effluent stressors which can correspond with exposure to municipal discharges. Further, these results provide a quantitative understanding of the importance of contaminants associated with effluent discharge (e.g., contaminants of emerging concern) on receiving water biological communities.

**RP064 Characterizing Exposures of Fish to Wastewater Treatment Plant Effluent: An Integrated Metabolite and Lipid Profiling Approach** D. Skelton, T. Collette, U.S. EPA, ORD, NERL, ERD; D. Martinovic-Weigelt, University of St. Thomas; G. Ankley, D. Villeneuve, U.S. EPA, ORD, NHEERL, MED; D. Ekman, U.S. EPA, ORD, NERL, ERD. Metabolite and lipid profiling are well established techniques for studying chemical-induced alterations to normal biological function in numerous organisms. These techniques have been used separately to successfully identify biomarkers of chemical exposure, screen for chemical potency, or to infer a chemical's toxic mode(s)-of-action. While metabolite and lipid profiling yield complementary data, the combined use of these techniques for biomonitoring has rarely been demonstrated to add value in chemical exposure assessment. Our group has recently investigated the efficacy of integrating

metabolite and lipid profiling data for exposure assessment by conducting biomonitoring studies in waterways near wastewater treatment plants (WWTP), using fathead minnows (*Pimephales promelas*). Caged fish were exposed to effluent from WWTPs as well as water from an upstream and downstream site in the same watershed. A variety of endpoints were measured in the fish, including targeted gene expression, NMR-based metabolite profiles, and GC-MS-based lipid profiles. We integrated the metabolite and lipid profiling data to better characterize the exposures of fish to the different sites and to yield a greater number of putative biomarkers of exposure. The results of our investigation demonstrate the utility of combining these complementary techniques for characterizing exposures of fish to chemical contaminants and identifying biomarkers of WWTP effluent exposures.

**RP065 Urban Stressors for Pesticide Endangered Species Assessments: Should Recent Nutrient TMDLs and Laws be Considered?** S.Z. Cohen, S. Haefner, L. Barnes, Environmental & Turf Services, Inc.. During 2000-2002, the US EPA proposed new, stringent nutrient criteria under the Clean Water Act (CWA). Pursuant to §303(d) of the CWA, state agencies have designated more than 41,000 of their surface water bodies as "impaired." Approximately 16,000 waters are impaired by nutrient-related pollution. Consequently, many TMDLs (total maximum daily loads) and state and local laws have recently been promulgated that target nutrients and the related parameters suspended sediments, dissolved oxygen, chlorophyll *a*, and temperature. Pesticide endangered anadromous fish assessments conducted by NOAA routinely list nutrients and temperature in urban and other environments as stressors that justify conservatism in their assessments. Full implementation of nutrient-based TMDLs would directly reduce the risk of eutrophication, which directly relates to dissolved oxygen concentrations. TMDL implementation can indirectly reduce urban runoff temperature increases as a result of vegetated BMPs. Thus these stressors can be reduced. A confounding factor is increased urbanization. Our initial focus on the Chesapeake Bay watershed and Florida is being expanded.

**RP066 Properly selected reference sites are needed to quantitatively attribute the loss of species to toxic pressure and other stress factors** D. De Zwart, RIVM / Lab. for Ecological Risk Assessment; L. Posthuma, RIVM / Lab. for Ecological Risk Assessment, RIVM / LER; S.D. Dyer, The Procter & Gamble Company / Central Product Safety, The Procter & Gamble Company / Miami Valley Innovation Center, The Procter & Gamble Company / Miami Valley Labs; K. Kapo, Montani Run, LLC; C.M. Holmes, Waterborne Environmental, Inc.. Selection of proper reference sites is critical to the diagnostic analysis of environmental monitoring data and thus environmental management. Regional reference conditions are commonly derived from relatively unimpaired sites within a homogeneous region and habitat type. After initial GIS-based selection, biological confirmation is periodically needed to validate site classification: disturbing stress factors may be neither readily visible nor detectable with the common screening methods used. In 2005 we published a study where we statistically attributed the effects of mixture toxicity and habitat alteration on the biological condition of fish assemblages in Ohio streams for the years 1990 – 1995. On average 50% of deviations from local biological reference were explained by the stress factors we had data on, including toxic mixtures. We hypothesized that addition of ecological stress factors could reduce unexplained biological variability. Therefore, we compiled a much more elaborate "golden Ohio dataset" for the years 2000 – 2007. Exploratory analysis of this dataset surprisingly revealed that the unexplained variance in the diagnosis increased as compared to the previous round. This was despite the fact that all additional predictors significantly contributed to the explanatory capacity of the attribution process. As an explanation for this, we suspected the presence of a reference condition problem. Of the 141 reference sites selected by Ohio EPA expert judgment, 57 reference sites had a more or less impaired biological condition, as indicated by low values (< 46) for the index of biological integrity (IBI) for the observed assemblages of fish. The mismatch between reference sites identified by expert judgment and biological condition on a specific sample moment could be largely attributed to local weather conditions that were significantly deviating from the long-term average. By restricting the set of reference data to expert identified sites that exhibited a good biological condition we were able to provide impact diagnostics. Amongst others, the observed impacts of toxic exposures in terms of the loss of species appeared to relate to the overall generic toxic pressure (multi substance potentially affected fraction of species – msPAF)



as predicted by toxicant concentration data, species sensitivity distributions (SSD) and considerations on mixture toxicity.

**RP067 Spatially-Relevant Statistical Tools to Ascertain Stressor-Biological Community Relationships at Variable Scales** K. Kapo, Montani Run, LLC; S.D. Dyer, The Procter & Gamble Company / Central Product Safety, The Procter & Gamble Company / Miami Valley Innovation Center, The Procter & Gamble Company / Miami Valley Labs; C.M. Holmes, Waterborne Environmental, Inc.; D. De Zwart, RIVM / Lab. for Ecological Risk Assessment; L. Posthuma, RIVM / Lab. for Ecological Risk Assessment, RIVM / LER. Stressor-response associations delineated by exploring spatial patterns between biological and other environmental variables in surface waters can provide useful screening-level hypotheses that can guide watershed, catchment and site-specific research and management strategies. A comprehensive database originally comprised of hundreds of environmental variables across the state of Ohio (2000-2007) was processed using a variety of variable reduction and selection techniques to generate a final selection of 30 predictor variables representing a range of habitat, chemical, toxicological, demographic, and other landscape factors from multiple agency resources linked to a National Hydrography Dataset-based hydrologic model. Random forest analysis on the final dataset provided initial stressor-response hypotheses at statewide and eco-regional scales, highlighting the role of habitat quality as a dominant determinant of aquatic community condition. Spatial patterns of environmental variables in the final dataset were further explored using GIS-based weights-of-evidence and logistic regression, which provided stressor-response hypotheses at the local catchment level. The utilization of a more robust variable selection process for model input provided an optimized representation of environmental factors which serves to improve both model quality and interpretation compared with previous "expert-driven" variable selection approaches.

**RP068 Transcriptional changes induced by organic chemical stress in soft coral, *Scleronephthya gracillimum*** S. Woo, Korea Ocean Research & Development / Southern Coastal Environment Div., Korea Ocean Research & Development Institute / South Sea Environment Research Dept.; S. Hwang, Ewha Womans University; S. Yum, Korea Ocean Research and Development Institute / Principal research scientist; J. Song, Ewha Womans University. The coral reefs, including soft coral communities, are known to be World's most valuable ecosystems in terms of ecological, economic and cultural capital but are in serious decline mainly due to the human-associated activities. Although the importance of the species has been continuously emphasized and management efforts have been successful locally, the worldwide decline of coral populations due to pollution, disease, and climate change is reaching a crisis. Over the last 30 years, coral reef assessment has provided an extensive description of certain responses at population and community levels in terms of coral cover, diversity and population dynamics of other reef species. However, with only these descriptive approaches for assessment are incapable of identifying the causes of deterioration of coral reef ecosystems. In this study the isolation of genes responding to persistent pollutants exposure in soft coral (*Scleronephthya gracillimum*) was described. Soft coral colonies were exposed to persistent organic pollutant, benzo(a)pyrene and heavy metals. Gene candidates whose transcript levels changed in response to chemical stress were identified by cDNA library construction. Twenty-five candidate genes were identified from benzo[a]pyrene stress exposed group, which are associated with cell cycle, cell signaling, transcription, translation, protein metabolism, and other cellular functions. Thirty-two candidate genes were identified heavy metals exposed group, which are associated with signal transduction, metabolisms, and protein turnover. The expected function of each gene was described. The isolated and identified differentially expressed genes have a potential to identify environmental stressors in environmental changes and could act as molecular biomarkers for biological responses against external environmental stimuli. The transcriptional changes of candidate genes were re-confirmed by real-time quantitative PCR (qRT-PCR). These isolated gene candidates were differentially expressed and the results suggested they could be used as molecular biomarkers for the identification of environmental stressors.

**RP069 The combined effects of calcium decline and predator kairomones on the gene expression of *Daphnia pulex*** I. Altshuler, University of Windsor, / Environmental Science; M. Cristescu, University of Windsor; H. Riessen, SUNY Collage Buffalo; N. Yan, York University. Freshwater organisms are constantly under pressure from an array of natural and

anthropogenic stressors. The complex interactions of these stressors can have surprising non-additive effects on individuals and populations. Here, we examined the combined effects of anthropogenic calcium decline and predator kairomones on the gene expression of *Daphnia pulex* and integrated the results with the physiological responses of the animals. Precisely, we quantified the expression of five *Daphnia* genes related to carapace building and calcium homeostasis in response to a gradient of four calcium concentrations (0.5, 1.5, 2.5 and 5.0 mgCa<sup>2+</sup>/l) in the presence and absence of *Chaoborus* kairomones. The five genes included ones coding for two cuticle proteins (*ICP1* and *ICP2*), sarco(endo)plasmic Ca<sup>2+</sup> ATPase (*SERCA*), inositol trisphosphate receptor (*IP3R*) and calbindin (*Calb32*). Overall, both stressors and their interaction significantly affected the expression of the genes in a non-additive manner. The decrease in calcium increased the expression of *ICP1*, *SERCA* and *Calb32* (7.3, 4.1, and 3.3 fold respectively) but only if the animals were simultaneously exposed to predator kairomones. The expression of *IP3R* increased with decreasing calcium concentrations 5.0 fold, but only in the absence of kairomones. The expression of *ICP2* was dependent on the specific calcium concentrations in the presence and absence of kairomones. Finally, results from the expression study were integrated with the physiological response of the animals to the combination of these stressors. Physiological responses included hardening of the carapace and formation of defensive structures.

**RP070 A Field Pilot Study Using Activated Carbon and Biochar to Reduce the Availability of PCDD/Fs in Soils/Sediments** Y. Chai, The Dow Chemical Company / Department of Environmental Chemistry, Louisiana State University; J.W. Davis, The Dow Chemical Company / Toxicology and Environmental Research and Consulting; R.J. Currie, The Dow Chemical Company / Toxicology, Environmental Research and Consulting; V. Fishman, G. Martin, The Dow Chemical Company / Environmental Analytical; M. Wilken, S. Lucas, D. Wandor, The Dow Chemical Company / Remediation; U. Ghosh, University of Maryland Baltimore County / Civil & Environmental Engineering, University of Maryland Baltimore County / Department of Chemical, Biochemical, and Environmental Engineering, University of Maryland Baltimore County / Chemical, Biochemical, and Environmental Engineering. A field pilot study was conducted to evaluate the activated carbon (AC) and biochar amendment as an in-situ remedial technology for soils/sediments impacted by polychlorinated dibenzo-*p*-dioxin/dibenzofurans (PCDD/Fs) from historical emissions traceable to incinerators and other sources. The total PCDD/Fs and toxic equivalent (TEQ) in the soil were 55.4 µg/kg and 394 ng/kg. The sorbents were mixed in the soil in the field at dose level of 0.2×soil total organic carbon content. The physicochemical availability of PCDD/Fs (TEQ) in soil was measured using polyoxymethylene (POM) passive samplers while the bioavailability was determined using terrestrial earthworm *E. fetida*. These measurements were conducted both in the laboratory and in the field at one month and one year after sorbent amendment in the field. Field and laboratory (numbers in parenthesis) measurements at one month post-application showed that the AC reduced the physicochemical availability and bioavailability by more than 64% (95%) and 76% (94%), whereas biochar by 37% (46%) and 0% (38%). The reduction efficiencies at 1 yr post-application were 69% (93%) and 59% (71%) for AC, whereas 9% (9%) and 11% (0%) for biochar. Results from this field pilot study showed that AC amendment is promising for in-situ treatment of PCDD/Fs in soils/sediment.

**RP071 Application of a Tenax Model to Assess Bioavailability of PCBs in Field Sediments** E.M. Mackenbach, Southern Illinois University; J. You, Guangzhou Inst Geochem, Chinese Academy Sciences / State Key Laboratory of Organic Geochemistry; M.A. Mills, U.S. EPA / National Risk Management Research Laboratory, ORD, US EPA / Remediation and Pollution Control Division, NRMRL, U.S. EPA / Office of Research & Development; P.F. Landrum, Southern Illinois University-Carbondale / Fisheries and Illinois Aquaculture; M. Lydy, Southern Illinois University Carbondale / Fisheries and Illinois Aquaculture Center and Department of Zoology. Bioavailability has been estimated in the past using equilibrium partitioning based biota-sediment accumulation factors. However, these values are not always reliable using field-collected sediments likely due to varying amounts of organic carbon particularly black carbon in sediments. Improved approaches to better evaluate contaminant bioavailability in sediment are needed. In the current study, a literature-based model was constructed that relied on both laboratory-exposed and field-collected oligochaete bioaccumulation data. The model system used 24 h Tenax



extraction data paired with bioaccumulation tests using oligochaetes to establish the ability and utility of the biomimetic extraction. The model was then tested to confirm its utility and reliability to estimate bioavailability of oligochaetes exposed to polychlorinated biphenyl (PCB) contaminated sediments from the Ottawa River, OH. The model correctly predicted the PCB bioaccumulation for 94.9% of all the congener data and 97% of the dioxin-like congeners. The mono- and di-substituted chlorinated biphenyls were the least well described, but the estimates were conservative, e.g., the model over-predicted bioaccumulation. Thus, the Tenax model was robust and reliable across a wide range of sediment characteristics for estimating PCB bioaccumulation in oligochaetes. This model has the potential to serve as an indicator of bioavailability and may be applied to measure remediation success without the use of extensive organism testing.

**RP072 Assessment of a Biochar Sediment Amendment using DGT**

D.D. Reible, The University of Texas-Austin, University of Texas / Environmental and Water Resources, The University of Texas at Austin / Dept. of Civil & Environmental Eng.; P. Bireta, The University of Texas-Austin / EWRE; R.C. Landis, DuPont Company / Engineering. The diffusive gradient in thin film (DGT) technique is applied to determine porewater mercury and methyl mercury concentrations in both laboratory and field studies. DGT devices have been a useful indicator of dissolved metals in aqueous systems, although the technique is not as well developed for measuring mercury or for measuring concentrations in sediments. Laboratory experiments showed that both resins strongly associated with dissolved  $Hg^{2+}$  and that the 3MP could be used to measure methyl Hg as well. Laboratory testing was used to evaluate mercury DGT probe uptake parameters from the sediment and to develop procedures for analysis and interpretation of the probe. DGT probes were then deployed in the field at a pilot pond near the South River (Virginia, USA) in order to measure the performance of a sediment amendment. Dissolved porewater mercury has been shown to better correlate with methylmercury production than bulk sediment mercury loading. Multiple disk probes and sediment probes were deployed in a pilot study pond in-situ to determine overlying water Hg concentrations and sediment porewater Hg and methyl mercury and concentration profiles over time. The initial deployments showed that the amendment is effective in reducing the bioavailable mercury concentrations and that the DGT could be a useful tool for exploring available and mobile mercury and methyl mercury in the field. Further examination of low biological productivity sediments is being conducted to see if the reduction of porewater mercury concentrations has the same effect on methylation.

**RP073 Bioavailability of Hydrophobic Organic Contaminants (HOCs) in Sediments Amended with Carbonaceous Materials and Sand**

J. Voelker, University of California, Riverside / Department of Environmental Sciences; X. Cui, University of California Riverside; W. Hunter, US Food & Drug Administration / Center for Veterinary Medicine, Food and Drug Administration / Center for Veterinary Medicine; J. Gan, University of California Riverside. Activated carbon and sand are often used in the remediation or capping of contaminated sediments. The effectiveness of these amendment practices is often evaluated by using the bulk or total sediment contaminant concentration. However, for strongly hydrophobic organic contaminants (HOCs) such as PCBs, DDTs, PAHs, and PBDEs, the total sediment concentration often is a poor indicator of the real risk of HOCs to non-target organisms such as benthic invertebrates, because the actual exposure is controlled by bioavailability. Therefore, bioavailability is an integral endpoint for assessing the toxicity of HOCs in sediments. We have carried out a series of studies at the bench scale and evaluated the effect of various carbonaceous materials including activated carbon, charcoal, biochar, and carbon nanotubes, and sand on the bioavailability of HOCs in sediments. Both bioassays and chemically based approaches have been applied to estimate the bioavailability of PAHs, DDTs, PBDEs, and pyrethroid insecticides. The chemically based methods included solid phase microextraction (SPME) and sequential desorption using Tenax. The results show that biodegradation potential or bioaccumulation into benthic invertebrates such as *Chironomus tentans* was better predicted by measurements such as the freely dissolved concentration ( $C_{free}$ ) or the rapid desorption pool ( $F_{rapid}$ ) than by the bulk concentration when sediments were amended with the different materials. Therefore, simple biomimetic methods such as SPME or Tenax extraction should be incorporated into the evaluation of remediation performance or effectiveness during sediment remediation and management.

**RP074 Comparison of Monitored Natural Attenuation Parameters**

**with Potentially Bioavailable Organic Carbon in Aquifer Sediments at Chlorinated Solvent Sites**

L.K. Thomas, Syracuse University / Department of Civil and Environmental Engineering; M.A. Widdowson, J.T. Novak, Virginia Tech / Department of Civil and Environmental Engineering; F.H. Chapelle, U.S. Geological Survey. Monitored natural attenuation (MNA) has become accepted by federal regulatory agencies as a viable remediation strategy for chlorinated solvents under favorable site conditions. At microbially-active chlorinated solvent sites, reductive dechlorination is typically the critical attenuation process in MNA-based remedial strategies. The efficacy of reductive dechlorination at sites with no anthropogenic carbon sources is often influenced by the availability of readily-biodegradable natural organic carbon along with favorable geochemical conditions for supporting microbial dehalogenation. A recent method was developed to quantify the pool of assessable natural organic carbon, operationally defined as potentially bioavailable organic carbon (PBOC). Evidence suggests that the level of PBOC, may be a key metric in predicting the longterm rate, extent, and sustainability of reductive dechlorination at MNA sites. In this study, relationships between PBOC and MNA parameters were investigated at chlorinated solvent sites. PBOC was measured using aquifer sediment samples collected at 12 study sites representing a wide range of environmental conditions. Using routine monitoring data, correlations were developed between PBOC, the rate and extent of reductive dechlorination, and site geochemical conditions. Direct relationships with PBOC were also developed between concentrations of hydrolyzable amino acids (HAA), proteins, and humic acids in aquifer sediment samples. Results demonstrated a statistically significant positive relationship between PBOC and first-order decay rates for total chlorinated ethenes ( $p < 0.05$ ). Consistent with these results, study sites with higher PBOC levels displayed lower levels of dissolved oxygen within the groundwater system. Greater concentrations of HAA, proteins, and humic compounds were also representative of study sites with higher levels of PBOC.

**RP075 Development of a success story for ecosystem health**

P. Michael, Natural Resources Canada, Geological Survey of Canada (Atlantic); G. Williams, D. Scott, Dalhousie University, Department of Earth Sciences; S. Mohamed, Dalhousie University, Department of Earth Sciences; F. Gagne, Environment Canada; A. Blouin, Water Halifax; T. Milligan, Fisheries and Oceans Canada; B. Law, Fisheries and Oceans Canada, Bedford Institute of Oceanography; J. Hellou, Bedford Institute of Oceanography / Ecosystem Research Division. In urban areas, the coastal zone receives both domestic and industrial effluents and is impacted to a variable degree depending on the continent, country and city under scrutiny. Efforts have been and are ongoing to understand and remediate the effects of population expansion on the environment. Laws and regulations do not always keep up with knowledge development because of the expense and long timelines often required to achieve successful changes. In Eastern Canada, relatively small populations with a large territory are the norm and many municipalities have been assessing the state of the coastal environment and implementing remedial action. The recent construction of wastewater treatment plants (WWTP) to improve the quality of combined sewage effluents represents a success story for Halifax, Nova Scotia, the largest city in Atlantic Canada. This study assessed the environmental quality of Halifax Harbor from 2008 to 2010, near the first of three planned WWTP relative to other sites, including two existing WWTP. Surface sediments and cores were collected using several sampling methods, i.e. slow cores, grab samples and vibrocores. Chemical analyses of sediments included steroid markers of particles in sewage, polycyclic aromatic hydrocarbons and metals. The health of deposited foraminifera, i.e. species diversity and deformities, was used to examine ongoing changes taking place relative to the historical and spatial coverage of the effluents entering this estuary. Monitoring of water quality included measurements of salinity, temperature, chlorophyll a, dissolved oxygen, metals, ammonia, total oil and grease and coliform bacteria. The results of this study showed that water quality improved rapidly following the opening of the new WWTP. Chemical conditions in bottom sediments have not changed substantially in the last 20 years, but the study did provide pre-industrial background levels for various contaminants that can be used to assess improvements in the future. Benthic foraminifera in surface sediments showed an increase in both diversity and abundance following the onset of sewage treatment. Societal pressure played a pivotal role in implementing constructive change and further improvements are on the horizon.

**RP076 Early Screening with Tissue Data to Improve Remedy Success** K. Godtfredsen, T. Deshler, Windward Environmental LLC. Reducing human health risks associated with seafood consumption is often a risk driver for sediment remediation under a variety of regulatory programs. However, site identification/delineation and contaminant of concern identification is often based on a comparison of sediment quality data to regulatory standards or background concentrations. Because human health risks are proportional to concentrations in tissue (rather than concentrations in sediment), there is a disconnect between data reviewed and goals identified, potentially resulting in costly sediment cleanups without reductions in human health risks. This talk presents an alternative paradigm for consideration in which tissue screening is conducted early in the process to determine: 1) whether incremental human health risks associated with a sediment area are unacceptable relative to background, and 2) which contaminants appear to be site-related in sediment in order to determine if the potential exists for sediment cleanup to result in a reduction in human health risks. This information provides a much firmer foundation for sediment remediation in locations identified because of human health concerns and also better manages expectations for remedy success.

**RP077 Growing Biofuel Crops on Abandoned Mine Lands** X. Zhao, / Ira. Fulton School of Engineering; A. Landis, Arizona State University; J. Monnell, University of Pittsburgh. Abandoned mine lands (AML) resulting from mining of coal are prolific in Pennsylvania with an estimated amount of 200,000 acres, and are often very acidic and contaminated with heavy metals such as As, Al and Cd. One common reclamation of AMLs includes re-vegetation of AML sites to reduce soil erosion and protect soils against degradation during reclamation. Growing biofuel crops on AML can create an opportunity to combine land remediation with biofuel production expansion. This talk investigates the viability of growing biofuel crops on AML. Phytoremediation and plant growth on coal refuse were assessed from experiments conducted in a greenhouse at the University of Pittsburgh. Five common biofuel crops, namely sunflower, soybean, canola, corn and camelina, were cultivated in a greenhouse environment on mine refuse from an abandoned coalmine located in Western Pennsylvania. Different natural and conventional soil amendments were investigated to neutralize the pH of the mine refuse. Crop growth under these varied conditions was monitored to determine the appropriate soil management strategy for biofuel crop production on AML. The level of contamination in the soil, plant matter, and leachate was analyzed before planting and after harvest with respect to nine major heavy metal contaminants through the use of Atomic Absorption Spectroscopy (AAS). Results from the greenhouse experiments show that soil amendments that neutralize acidic mine refuse are a necessary step for enabling plant growth and providing nutrients in the soil amendments can significantly improve the plant growth on mine refuse to a comparable level with that on the regular garden soil. Elevated metal concentrations were found in all plants grown on soil containing mine refuse. The concentrations of Al, Fe, As, Cr, Cd and Se in water samples collected from non-vegetated mine refuse were determined to far exceed the maximum contaminant levels given by Pennsylvania Department of Environmental Protection. But, the addition of soil amendments and plant growth could effectively reduce the amount of contaminants that enter runoff. This research provides evidences that growing biofuel crops can be a useful management for AMLs and an opportunity for valuable use of these degraded lands.

**RP078 Low-impact delivery of sorbent amendments to reduce contaminant bioavailability in sediments** U. Ghosh, University of Maryland Baltimore County / Civil & Environmental Engineering, University of Maryland Baltimore County / Department of Chemical, Biochemical, and Environmental Engineering, University of Maryland Baltimore County / Chemical, Biochemical, and Environmental Engineering; C. Menzie, Exponent, Inc., Exponent / EcoSciences; S. Kwon, University of Maryland Baltimore County; B. Amos, Exponent. In-situ treatment with low-impact delivery of activated carbon can be especially attractive for PCB-impacted vegetated areas and sensitive wetlands. This study was performed at USEPA Superfund site contaminated wetland and a creek that flows into the James River in Virginia. This research is aimed at developing the in-situ remediation technology through laboratory and pilot-scale testing and addresses the critical barriers in the advancement of the technology. Initial laboratory studies demonstrated reduction in the bioavailability of PCBs and a range of chlorinated pesticides in sediment after amendment with activated carbon. Pilot-scale control and treatment sites were 15meters by 15meters,

half within the channel segment and half in the bordering marsh. Activated carbon was formed into pellets named SediMite and was applied using a boat-mounted dispersion device similar to a commercial fertilizer/herbicide spreader. The treatment was deployed in the summer of 2009. Samples were collected and examined before treatment, two months, and 16 months after treatment. Black carbon measurement in sediment cores demonstrated that the applied activated carbon remained in sediment after application and was found in the top 5 cm of sediment. Bioaccumulation studies using the benthic organism *Leptocheirus plumulosus* as well as aqueous concentration measurement using a passive sampler showed reductions in PCBs at the treatment sites after deployment. Benthic community sampling after application demonstrated no significant impact of the application on native biota. A summary of the results from this test site and other ongoing pilot studies of the technology will be presented.

**RP079 Mapping Risk to Predict Risk Reduction from Remedial Design Alternatives for Contaminated Sediments in the Patapsco River, Baltimore Harbor, Maryland** M. Ciarlo, EA Engineering, Science, and Technology, Inc., EA Engineering, Science, and Technology; M.W. Powell, EA Engineering, Science & Technology / Water Resources Department; P. Derrick, EA Engineering, Science and Technology, Inc. / Department of Water & Natural Resources; C. Papegeorgis, EA Engineering, Science, and Technology, Inc.; M. Slatnik, Maryland Environmental Service; M. Simon, Maryland Environmental Service; S. Storms, F. Hamons, Maryland Port Administration. The Maryland Port Administration (MPA) is interested in acquiring the Coke Point Peninsula (Coke Point) on Sparrows Point as a potential site for a Dredged Material Containment Facility (DMCF) that may utilize or cover near-shore areas within the Patapsco River. Planning studies found that sediments contain elevated concentrations of metals, polycyclic aromatic hydrocarbons, and polychlorinated biphenyls. One of the potential benefits of a DMCF which extends into the river is that it may cover over areas of elevated concentrations and thus reduce risks to people, wildlife, fish, and crabs from legacy pollutants. Therefore, risk assessment and management studies were performed to quantify the beneficial risk reduction associated with the DMCF. Analytical results from sediment sampling were kriggered to extrapolate distribution of chemical concentrations across the study area for over 1000 grid cells. Risk estimates for individual grid cells were calculated using human health and ecological risk models. Cumulative hazard indices for humans, wildlife, benthos across cells were used to create risk surfaces as a conceptual model for site. Over 30 possible remedial scenarios were mapped over risk surfaces, and risks were recalculated based on assumptions regarding the effect of each scenario on grid cell risk levels. DMCF concepts achieve over 80% risk reduction for humans, over 20% for wildlife, and over 70% for benthos. Predicted risks after remediation were compared to background risks and regulatory standards. Graphical representations based on risk surfaces played an important role in planning and risk communications and have been a key factor in preparing feasibility and corrective measures studies.

**RP080 Analytical methods for the pharmaceuticals and personal care products in the tissues of raptors** R. Tanoue, Ehime University / Center for Marine Environmental Studies (CMES); K. Nomiya, Ehime University; H. Nakamura, R. Shinohara, Prefectural University of Kumamoto / Graduate School of Environmental and Symbiotic Sciences; S. Tanabe, Ehime University / Center for Marine Environmental Studies. Pharmaceuticals and personal care products (PPCPs) have been detected in the aquatic environment such as surface water, groundwater and even drinking water. PPCPs are constantly excreted by human beings and their contaminations are widespread in the environment and wildlife and also known to affect endocrine systems. Recently, it was reported that diclofenac is a principal cause of a dramatic decrease of endemic vulture species in India, Pakistan, and Nepal. Diclofenac is one of the non-steroidal anti-inflammatory drugs (NSAIDs) used for the treatment of inflammation, fever, and pain in human beings and domestic livestock. Anti-inflammatory effect of NSAIDs is based on a prostaglandin synthetase inhibition. As a result, it was reported that diclofenac causes strong renal insufficiency to vulture species. Higher trophic level species such as raptors (including vulture species) play an important role in the sustainability of ecosystems. However, investigations on raptors are limited to the Indian subcontinent, and information on contamination by the NSAIDs on raptor species from other parts of the world is scarce, although bioaccumulation levels and potential negative effects vary among species. It is therefore necessary to investigate the occurrence and ecological

risk of NSAIDs to raptor species by a comprehensive monitoring survey. The aims of this study were to develop analytical methods for NSAIDs and other PPCPs in the kidney and liver tissues of raptor species, and to conduct a pilot scale monitoring study. Extraction and clean-up procedures (using gel permeation and silica gel chromatography) were optimized and validated. These methods are capable of measuring PPCPs including NSAIDs at environmentally relevant concentrations in the kidney and liver tissues of raptor species. In the pilot study, presence and residual levels of PPCPs in raptor and other avian species collected from across Japan were measured. Predictably, several target PPCPs including N,N-diethyl-m-toluamide (insect repellent) and triclocarban (antibacterial agent) were detected in the avian tissues.

**RP081 Application of a decision tree tool to the evaluation of health risks from combined exposures to compounds in European effluents and surface waters** P.S. Price, The Dow Chemical Company / Toxicology & Environmental Research & Consulting; C. Watts, Chris Watts Associates Ltd; D. Leverett, WCA Environment Limited. A decision tree for the assessment of the risks posed by combined exposures to multiple chemicals has been developed by the Cefic Mixture Industry Ad Hoc Team based industry sponsored research on mixtures, WHO guidance on tiered approaches for assessing mixtures, and the recent EU report *Toxicity and Assessment of Chemical Mixtures*. The decision tree provides a systematic approach for organizing information concerning the exposure to multiple chemicals and their toxicity and uses a screening approach to focus on co-exposures that are the greatest concern. The decision tree organizes cumulative exposures into three groups, exposures that are a concern because exposure to one or more compounds are a concern, exposures of low concern for cumulative effects, and exposures where exposures to chemicals do not pose a concern but where cumulative exposures are a concern. The tool also identifies chemicals where information on mechanism of action is most necessary to refine the assessment. The tree was used to evaluate human health effects from the combined exposure to 559 mixtures of substances measured in surface waters and effluents discharged to surface waters. The substances included metals, pesticides, pharmaceuticals, and chemical components of consumer products. A total of 222 substances were detected in one or more samples and the number of detected substances in any one sample ranged from 2 to 49 substances. The key findings were, 1) using conservative exposure and toxicity assumptions 550 of the 559 mixtures were found to be of low concern, 2) the majority of the toxicity came from a single chemical in 44% of the exposures, and 3) the 9 mixtures where risk from combined exposures was a concern, would have been identified by chemical-by-chemical assessments. In summary the decision tool was able to provide useful information on the potential for the occurrence of cumulative risks from mixtures of chemicals discharged to surface waters.

**RP082 Assessment of Glucocorticoid Concentrations In Wastewater Influent and Effluent for the Paso Del Norte Region** M.C. Lozano, University of Texas at El Paso / Chemistry; W. Lee, University of Texas-El Paso. Glucocorticoids are an understudied class of steroid hormones, widely prescribed in the United States for their anti-inflammatory and immunosuppressive properties. Glucocorticoids have been implicated in the advent of various health conditions in humans—such as psychological and metabolic disorders. Glucocorticoids are widely prescribed and used in the United States for a variety of ailments and diseases. Thus, large population centers are likely to contribute significantly to the amount of glucocorticoids released into the environment. The Paso del Norte region (Las Cruces, NM; El Paso, TX; and Ciudad Juarez, Mexico) not only has a large human population but also has limited surface and ground water resources. Recycling of treated water is becoming a necessary practice for this semi-arid areas, and water pollution and management of glucocorticoids must be addressed for the protection of human health and the environment. The proposed research will evaluate bi-national water pollution in order to quantify the presence of glucocorticoids to assess the integrity of the treated water and potential problems in water management. Through liquid chromatography-mass spectrometry (LC-MS) and an established 4-hour yeast bioassay, presence, concentrations, and bioactivity will be assessed in wastewater samples.

**RP083 Comparing the fingerprint of halogenated organic compounds in two ecotypes of Southern California bottlenose dolphins using non-targeted analysis** N.J. Shaul, University of California San Diego / Scripps Institution of Oceanography; E. Hoh, San Diego State University; N.

Dodder, Southern California Coastal Water Research Project; L. Aluwihare, University of California San Diego; K. Maruya, Southern California Coastal Water Research Project; D. Weller, NOAA / Southwest Fisheries Science Center. Searching for unknown chemicals that are outside of the standard list of pollutants (i.e., PCBs, PBDEs, pesticides, etc.) requires a non-targeted analytical approach. There are documented differences in the halogenated chemical fingerprint (i.e., the abundance pattern of the suite of halogenated chemicals in a given sample) depending on the sample matrix, level of perturbation or exposure, and/or the trophic level of the organism sampled. The objective of this study is to assemble a library of the halogenated organic compounds observed in southern California bottlenose dolphins (*Tursiops truncatus*) that includes mass spectra and related identification information. In addition, the semi-quantitative abundances of each compound relative to an internal standard will be catalogued for individual organisms and each habitat. Blubber samples (2 g) from mature male dolphins, corresponding to coastal and offshore ecotypes of *T. truncatus* from southern CA, were extracted and analyzed by two dimensional gas chromatography and time of flight mass spectrometry (GCxGC/TOF-MS). For the initial dolphin sample, chosen at random from the pool of samples from both ecotypes, the LECO ChromaTOF® software identified a total of 17,038 peaks at a signal-to-noise ratio of 10 and searched against NIST's 2011 mass spectral library. The spectra of each peak was then scrutinized to identify the halogenated compounds in the sample by recognizing the halogenation patterns for Br and Cl. The reference library resulting from this work will be used to identify and compare bioaccumulative contaminants in other environmental samples, including sediments, fish, and other marine mammal species (including *Tursiops*).

**RP084 Contaminants of Emerging Concern in Source and Treated Drinking Waters: Temporal Analysis of Presence, Concentration, and Qualitative Removal** S.T. Glassmeyer, U.S. EPA / Office of Research and Development, U.S. EPA / NERL/MCEARD/CERB; E.T. Furlong, U.S. Geological Survey / National Water Quality Laboratory; D. Kolpin, USGS; A.L. Batt, U.S. EPA Office of Research and Development / National Exposure Research Laboratory, US EPA; B. Benson, USEPA / Region 8; S. Boone, U.S. EPA / Office of Chemical Safety and Pollution Prevention, USEPA/ECB / USEPA/OCSP/OPP/BEAD/Environmental Chemistry Laboratory; O. Conerly, USEPA / Office of Water; M. Donohue, USEPA / Office of Research and Development; H. Mash, U.S. EPA / Office of Research and Development, NRMRL; S. Pfaller, K. Schenck, J. Simmons, E. Varughese, S. Vesper, E. Villegas, M. Ware, V. Wilson, USEPA / Office of Research and Development. The US Environmental Protection Agency (USEPA) and the US Geological Survey (USGS) have collaborated on two sampling campaigns assessing untreated and treated drinking water sources in the United States. In Phase I (2007), samples from nine drinking water treatment plants (DWTPs) were analyzed for 83 contaminants of emerging concern (CECs). In Phase II (2010-2012), samples from 25 DWTPs were analyzed for over 250 analytes (which includes the 83 Phase I analytes). Five DWTPs were sampled in both Phase I and II. Three of the Phase I DWTPs used advanced treatment techniques such as ozone/chloramine, granular activated carbon beds, and UV irradiation. One DWTP used conventional treatment (coagulation, sedimentation, filtration, chlorination). The final location was a groundwater DWTP that only chlorinated the water. This poster examines the trends in analyte presence, concentration, and qualitative removal from these five sources during the two sampling campaigns.

**RP085 Effect-directed analysis (EDA) of Elizabeth River Porewater: Developmental Toxicity in Zebrafish (*Danio rerio*)** M. Fang, G. Getzinger, P. Lee Ferguson, H. Stapleton, Duke University. The Elizabeth River (ER) estuary (Virginia, USA) includes the Atlantic Wood Industries Superfund Site (Portsmouth, VA, USA). Zebrafish (*Danio rerio*) exposed to porewater collected from the Elizabeth River sediment near this Superfund site exert acute toxicity and cardiac teratogenesis, presumably caused by high sediment levels of polycyclic aromatic hydrocarbons (PAHs) from old creosote use. However, the specific chemicals and/or mixtures primarily responsible for this toxicity have not been clearly identified. In order to identify the compound(s) that are likely causing this toxicity, an effect-directed analysis (EDA) using zebrafish embryos is being conducted. Endpoints used for assessing toxicity include mortality, malformation rate and pericardial area. In this study we examined toxicity of porewater that was processed using several different methods to compare toxicity in particulate bound and dissolved phase fractions. Raw porewater was collected from sediments by



centrifugation and then further processed several different ways to examine toxicity of the dissolved and particle/colloidal phases. Porewater processing included high speed centrifugation (288,000 x g, 3h), low speed centrifugation (~3000rpm, 20mins), and physical filtration (~0.7  $\mu$ m GFF). Particles collected from the samples centrifuged and from the GFF filters (organic extracts) were re-suspended in purified water and assessed for toxicity alongside the raw and dissolved phase porewater fractions. The LC50 dose-response relationship was compared among treatments and revealed that the GFF filtered particles showed comparable toxicity with raw porewater. The next most toxic fraction was the sample prepared from resuspension of particles isolated from the low speed centrifugation, followed by filtered porewater. The porewater after low speed centrifugation could recover ~70% of the original toxicity based on calculation of pericardial area. In contrast, the porewater which had undergone ultracentrifugation did not show any obvious toxicity. Furthermore, the malformation rate and the pericardial area would decrease by a factor of 2 after GFF filtration. The result of this study indicated that the particulate phase of ER porewater, especially the colloids in the porewater played a great role in the toxicity. The particle phases are now being extracted, fractionated using liquid chromatography and analyzed using high resolution mass spectrometry to try to identify the most toxic components.

**RP086 Evaluation of Surface Waters Associated with Animal Feeding Operations for Estrogenic Chemicals and Activity** N.W. Shappell,

USDA-ARS-RRVARC / Biosciences Research Lab, USDA-ARS; D.A. Alvarez, D.W. Kolpin, W.T. Foreman, J.L. Gray, M.T. Meyer, USGS. Estrogens and estrogenic activity (EA) were evaluated in surface waters associated with animal feeding operations. Water was sampled at 19 sites in 12 states using discrete (n=41) and POCIS (n=19) sampling methods. Estrogenic chemicals measured in unfiltered water by GC/MSMS included: estrone (E1), 17 $\alpha$ - and 17 $\beta$ -estradiol ( $\alpha$ - and 17 $\beta$ -E2), estriol (E3), and ethinylestradiol (EE2). EA was evaluated in SPE extracts of filtered water by EScreen, and the Yeast Estrogen Screen (YES). Water from 7 sites contained estrogens and EA (YES and EScreen). E2 Equivalent (E2Eq) ranges were 0.006–0.49 ng/L, GC/MSMS; 0.06–0.35 ng/L by EScreen, except for a manure spill-event sample (63 ng/L GC/MSMS, 42 ng/L EScreen E2Eq). GC/MSMS detected estrogens in 3 samples that were non-detects (NDs) by YES, two of those also EScreen NDs. Sample toxicity was found in one of the EScreen NDs. EScreen sensitivity (LOQ ~0.017ng/L E2Eq) allowed for detection of EA in water from all sites (0.03–0.22 range; field blank 0.019 ng/L, n=10). Estrone was the most abundant and frequent estrogen detected by GC/MSMS, but the proposed No Effect Concentration (pNOEC) for 17 $\beta$ -E2 in fish of 1 ng/L was not exceeded except in the post-manure spill samples. For 11 POCIS samples, all assays detected presence of estrogens or EA. Three of the 4 highest concentration samples were from streams accessed by grazing cattle; one of these neared the NOEC for 17 $\beta$ -E2 (0.9 ng/L GC/MSMS E2Eq, 0.6 ng/L EScreen E2Eq). The 4<sup>th</sup> sample was from a drainage ditch in a region where 80% of the basin received poultry manure applications. EScreen and YES identified 4 POCIS samples as estrogenic although the estrogens were NDs by GC/MSMS. YES did not detect 3 positive POCIS samples based on GC/MSMS and EScreen data. These ND samples contained  $\leq$ 0.13 ng E2Eqs after a >40 day deployment. While EE2 was never found, estrone was detected in 13 POCIS samples. Unlike 17 $\beta$ -E2, estrone was always present, and at higher concentrations, whenever any other estrogen was detected in water or POCIS, making it a good marker compound for estrogen contamination. While grazing cattle were associated with water having the highest EA, discrete post-manure application runoff samples may have been higher, if sampled at different times. Study results indicate YES to be a relatively inexpensive, adequately sensitive tool for identification of EA at environmentally-relevant concentrations on SPE-extracted water or POCIS samples.

**RP087 Identifying pharmaceuticals in the effluent of a southern Illinois wastewater treatment facility by liquid chromatography – tandem mass spectrometry** M. Siebert, Southern Illinois University Edwardsville /

Environmental Sciences Program; K.A. Johnson, Southern Illinois University at Edwardsville / Department of Chemistry & Environ. Sciences, Southern Illinois University Edwardsville / Chemistry & Environmental Sciences, Southern Illinois University Edwardsville / Department of Chemistry & Environmental Sciences Program. Water quality and safety continues to be of epic concern, partly due to the revelation of pharmaceutical residues in various waterways. Human ingestion followed by excretion into municipal

wastewaters represents the initial, primary pathway of environmental release. Standard wastewater treatment (WWT) facilities do not efficiently remove pharmaceuticals; therefore, wastewater effluent signifies the prominent vehicle for aquatic contamination. Even though acute (primarily) and chronic aquatic ecotoxicity data have been established for many pharmaceuticals, the EPA does not currently require WWT facilities to monitor their presence in the influent or effluent. Despite the global prevalence of literature on pharmaceutical presence in wastewater effluents, this data is scarce in Southern Illinois. The focus of this study was to identify pharmaceuticals in a southern Illinois WWT facility's effluent, review pertinent regulations and investigate community pharmaceutical waste disposal practices to establish baseline data, assisting in WWT process improvements and future research. Sixteen compounds were selected for screening during 10 randomly timed effluent sampling events. Samples were extracted in duplicate using Oasis® HLB solid phase extraction. Analysis of the extracts was performed on a Varian 310 LC-MS/MS employing Electrospray Ionization (ESI) in both positive and negative modes. The reporting levels were either 10 or 50 parts per quadrillion (pg/L), depending on the compound. Of the 16 compounds, 8 were identified in the effluent in the ng/L to ug/L range. Five of those 8 compounds were detected in all 20 samples. These included Carbamazepine, Carbamazepine epoxide, DEET, Diltiazem, Fluoxetine, Ketoprofen, Methyl paraben and Sertraline. Carbamazepine had the highest concentration detected (0.55 ug/L) and Fluoxetine had the lowest (5 ng/L).

**RP088 Influence of *Afromomum melegueta* seed oil on biomarkers of prostate and cardiac dysfunction** M.I Akpanabiutu, N.D. Ekpo, E.J. Akpan, University of Uyo / Biochemistry. *Background/Aim:* *Afromomum melegueta* is popular medicinal plant in Nigeria believed to be essential for the effectiveness of traditional medicine. This study aimed to determine the acute toxicity, phytochemical of this plant and its effects on lipid profile, haematological indices and biomarkers of prostate and cardiac dysfunction. *Materials and Methods:* Twenty four Wistar rats (144-326g) were used in four groups of six animals. Group I (control) received normal saline; groups II, III and IV, received intra-peritoneal injection of 27.39, 54.77 and 82.16 mg/kg body weight of the extract respectively for seven days. Haematological and biochemical parameters were measured. *Results:* Alkaloids, flavonoids, saponins, tannins, cardiac glycosides, terpenoids and steroids were identified. The LD<sub>50</sub> was 273.86 mg/Kg body weight. PSA decreased significantly in group II. Testosterone increased significantly in all the test groups compared to the control. Cardiac troponin I (0 ng/dl) was recorded for the test groups while the control had 1.69  $\pm$  0.12 ng/dl. Lipid profile results showed increase in HDL and decrease in Total cholesterol and LDL-Chol. Hemoglobin (Hb) and Red Blood Cells count (RBC) decreased significantly in group IV. WBC count, MCV, MCH and MCHC did not change significantly. *Conclusion:* *Afromomum melegueta* seed oil has the potential of ameliorating benign prostatic hyperplasia (BPH) and cardiac dysfunction as indicated by testosterone, PSA, lipid profile troponin I levels. Low Hb, RBC and LD<sub>50</sub> of 273.86 mg/kg bd. wt. are indicative of toxicity.

**RP089 Investigation of the degradation of endocrine disrupting compounds in aqueous matrices** T. Carrick, University of Texas-El Paso /

Department of Chemistry; W. Lee, University of Texas-El Paso. Throughout the recent decade, endocrine disrupting compounds (EDCs) have emerged as compounds of interest due to their negative, including both developmental and reproductive, effects observed on aquatic organisms. EDCs can be either naturally occurring, or manmade, and have been proven to enter the environmental water supply anthropogenically. Studies have identified one such source as municipal waste water treatment plants, where EDCs are present in both the influent waste water, as well as the effluent. However, with factors such as biodegradation, hydrolysis, and photolysis, the exact concentration of EDCs present in a water sample at the time of sampling versus the time of analysis is difficult to determine. Additionally, this makes estimating the concentration of EDCs being released into the environment through waste water treatment plants equally trying. Therefore, the aim of this study is to determine the stability of EDCs in different aqueous matrices by quantifying the rate at which they degrade. Degradation studies will be performed at different sample storage temperatures, as well as water pHs. EDCs tested are bisphenol-A, nonylphenols, 4-tert-octylphenol, estrone, and estradiol. Data of EDC degradation in waste water influent, waste water effluent, river water, as well as laboratory deionized water will be presented.

**RP090 Isolation and identification of ligands for the goldfish testis androgen receptor in condensates from a Canadian bleached kraft pulp and paper mill** P.D. Scott, Griffith University / School of Environment; C. Milestone, Wilfrid Laurier University / Department of Biology; S. Smith, Wilfrid Laurier University / Department of Chemistry, Wilfrid Laurier University / Chemistry; D. MacLachy, Wilfrid Laurier University / Canadian Rivers Institute, Wilfrid Laurier University / Department of Biology; M. Hewitt, Environment Canada / National Water Research Institute. This study builds on a series of investigations characterizing substances in kraft mill chemical recovery condensates that depress sex steroids in fish. Here, incubations of goldfish testis androgen receptors (AR) with condensate extracts were used to investigate the potential role of androgens in hormone depressions. Condensates contained variable levels of AR ligands, with the highest amounts in nonpolar extracts of filtered solids prior to solid phase extraction (SPE). High pressure liquid chromatography (HPLC) fractionation recovered the majority of activity in one fraction, with ligands detected in three additional fractions. Gas chromatography mass spectrometry analysis of the most active fraction confirmed the two most abundant components as the diterpenes manool and geranyl linalool. Manool exhibited a relative affinity for the AR that was 300 fold less than testosterone and accounted for 26% of total filtered solids activity. Geranyl linalool exhibited no affinity for the AR. Three additional diterpenoid families were tentatively identified as principal components of the three other androgenic HPLC fractions. Compared to condensates, final effluent had 3000 fold less androgenic activity, with < 1% attributable to manool. Putative androgens previously associated with mill effluents (androstenedione and androstadienedione) and progesterone were not detected; however, additional condensate diterpenes suspected as androgens were identified in final effluent. This study is the first to confirm nonsteroidal cyclic diterpenes as androgenic at pulp mills. A major in-mill source of these substances was identified, and the role of androgens in mill effluents affecting fish reproduction was reinforced.

**RP091 Macrophytes and nutrients in model sewage treatment wetlands do not influence pharmaceutical fate** P. Cardinal, University of Manitoba; J. Carlson, J. Low, University of Winnipeg / Richardson College for the Environment; M. Hanson, University of Manitoba / Department of Environment and Geography, University of Manitoba / Faculty of Environment; C.S. Wong, University of Winnipeg / Richardson College for the Environment, Richardson College for the Environment. In many areas treatment wetlands are used as a polishing step for municipal effluents, especially in smaller rural communities. In light of increasing regulatory oversight of effluent release on the Canadian Prairies, these systems are likely to become more popular as they are low-cost to install and maintain relative to more intensive treatment options. Pharmaceuticals are ubiquitous contaminants in these effluents, and so treatment wetlands would ideally be able to remove them prior to release into receiving waters. We examined the role of macrophytes in enhancing pharmaceutical removal in shallow model treatment wetlands (mesocosms) in two studies in 2011. In the first study, replicated wetlands were established with and without macrophyte communities (emergent, submergent, and floating plants) and dosed with six pharmaceuticals (carbamazepine, clofibric acid, naproxen, fluoxetine, sulfapyridine, sulfamethoxazole) in a synthetic wastewater matrix to simulate a pulsed release. There were no significant differences in the calculated half-lives between the two systems. In the second study, the same compounds were introduced into systems that contained macrophytes, with and without a phosphorous addition in order to stimulate primary productivity. Again, there were no significant differences in the compounds' half-lives between the two scenarios. No toxicity to macrophytes was evident in either experiment. From our studies, we conclude that the presence or absence of macrophytes do not significantly influence the fate of pharmaceutical contaminants in treatment wetlands. Dissipation processes (e.g., photolysis, sedimentation) were likely not influenced by the presence of macrophytes.

**RP092 Occurrence and Removal Mechanism of Pharmaceuticals, Pesticides and EDCs in the Water Treatment Plant** S. Nam, B. Cho, Y. Choi, Seoul National University; K. Zoh, Seoul National University, Seoul National University / Department of Environmental Health, School of Public Health. Occurrence of emerging pollutants such as pharmaceuticals, endocrine disruptors, and pesticides in surface waters has been the issues and widely evaluated recently. The presence of such organic contaminants might have the negative impacts on the quality of drinking water together with unknown toxicological effects through chronic exposure. Therefore, the

incidence of these emerging pollutants in raw waters used for drinking water production and their elimination through treatment must be considered in terms of health safety for water production. In this study, the influent and effluent samples passing through the selected water treatment plant (WTP) in Seoul, Korea. The samples were obtained mainly during summer and winter seasons which has different turbidities ranging from 3–680 NTU. Total 14 contaminants including 9 pharmaceuticals (acetaminophen (ACT), caffeine (CFF), carbamazepine (CBM), diclofenac (DCF), ibuprofen (IBU), naproxen (NPX), metoprolol (MPT), sulfamethoxazole (SMZ), and sulfamethazine (SMA)), 2 pesticides (atrazine (ATZ), 2,4-dichlorophenoxyacetic acid (2,4-D)), and 3 endocrine disruptors (bisphenol-A (BPA), nonylphenol (NP), and triclocarban (TCB)) were monitored using LC-MS/MS. Among 14 contaminants, ACT, CFF, SMZ, SMA, MPT, CBM, IBU, NPX, NP, TCB were observed in the WTP's influent and effluent with the range of 1.86 ~ 4,845 ng/L. Especially, the levels of 4 pharmaceuticals including ACT (2,541 ng/L), CFF (130 ng/L), DCF (206 ng/L), and IBU (203 ng/L) were higher in the influent. The concentration of ACT was the highest since it is widely used as a veterinary and human anti-inflammatory drug in Korea. The levels of most pollutants were higher in turbid water samples especially in rainy season which might be due to the discharge of wastewater and agricultural runoff. The removal efficiencies of these pollutants in WTP were examined by comparison the levels of influent and effluent of each treatment process. Average removal efficiencies after coagulation process were higher than 40% except CBM (15.7%), NP (23.23%) and MPT (23.9%). Especially, the removals of TCB (88.8%), CFF (83.1%) and SMZ (85.4%) were higher. It was also found that photo-degradation is the dominant mechanism in the removal of CFF and ACT, and the adsorption was the dominant removal mechanism for hydrophobic pollutants ( $\log K_{ow} > 2.5$ ) such as NPX, IBU, DCF, NP, TRB and BPA.

**RP094 Occurrence of PPCPs and hormones in South Florida drinking water** C. Wang, Florida International University / Chemistry Department; P. Gardinali, Florida International University / Department of Chemistry and Biochemistry. The quality of drinking water is a public concern since any compound present in the drinking water enters the human body directly. An online solid phase extraction (SPE)-ultra performance liquid chromatography (UPLC)-heated electrospray ionization (HESI)-tandem mass spectrometry (MS/MS) method and an online SPE-UPLC-atmospheric pressure photoionization (APPI)-MS/MS method were used to detect 72 PPCPs and hormones in drinking water samples. A Hypersil Gold aQ column (20 mm × 2.1 mm, 12 µm particle size) from Thermo Scientific was used to concentrate target compounds from drinking water samples. A Hypersil Gold aQ column (50 mm × 2.1 mm, 1.9 µm particle size) from Thermo Scientific was used to separate the target compounds. A heated electrospray source (HESI) was used to ionize polar target compounds and a photoionization source (APPI, Syagen Technology) was used to ionize less polar target compounds. A TSQ quantum Access tandem MS QqQ (Thermo Scientific) was used for compound detection using at least 2 SRM transitions. This study presents results from a comprehensive survey of 72 compounds in 54 tap water samples collected from the metropolitan Miami-Dade area. A total of 15 compounds were detected in the drinking water samples and at least one compound was detected in 93% of samples. The maximum concentration of salicylic acid, ibuprofen and DEET were 521 ng/L, 301 ng/L and 290 ng/L, respectively. The three most frequently detected compounds were DEET (93%), carbamazepine (43%) and salicylic acid (37%), respectively. Results indicated that drinking water sources, such as ground water, are possibly influenced by discharge of wastewater, disposal of biosolid from WWTPs or landfilling or intrusions of surface or treated waters into the distribution system. Current drinking water treatments cannot prevent certain PPCPs, such as DEET and carbamazepine, from entering the drinking water system.

**RP095 Occurrence of veterinary antibiotics in pen material and airborne particulate matter originating from beef cattle feedyards** A.D. McEachran, Texas Tech University / The Institute of Environmental and Human Health, Environmental Toxicology; B.R. Blackwell, Texas Tech University/TIEHH / Environmental Toxicology, Texas Tech University / Environmental Toxicology; K.J. Wooten, Texas Tech University / TIEHH; P.N. Smith, Texas Tech University / Environmental Toxicology, Texas Tech University / Institute of Environ. & Human Health. Large scale commercial beef production in the United States relies on extensive use of veterinary pharmaceuticals to treat disease and promote growth. Antibiotics are

frequently administered at sub-therapeutic doses to achieve both objectives and enhance production efficiency. Veterinary antibiotics have been detected in feedyard runoff and in land-applied manure at concentrations that inhibit microbial activity in soil and aquatic environments, and that facilitate the development of antibiotic resistance in bacteria. The objective of this study was to determine concentrations of the veterinary antibiotics tylosin and monensin in feedyard pen material and airborne particulate matter emanating from beef cattle feedyards to evaluate potential aerial entry of these compounds into the environment. Feedyard pen material and total suspended particulate matter samples were collected from feedyards across West Texas and analyzed via LC-MS/MS following liquid-solid extraction and solid-phase extraction (SPE) clean-up. Concentrations of tylosin and monensin reached high  $\mu\text{g}/\text{kg}$  levels in both pen material and particulate matter samples from multiple feedyards. This study indicates that transport of veterinary antibiotics into the environment via windborne particulate matter does, in fact, occur. These data highlight the need for subsequent studies which will determine the extent to which antibiotics used as growth promoters at beef cattle feedyards facilitate the development and spread of antibiotic resistance.

**RP096 Photodegradation kinetics and Mechanism of Bisphenol-A in water** Y. Kang, S. Nam, J. Im, Seoul National University; K. Zoh, Seoul National University, Seoul National University / Department of Environmental Health, School of Public Health. Bisphenol-A (BPA), known as an endocrine disrupting compounds (EDCs), is hardly removed in conventional wastewater treatment processes. In this study, we investigated kinetics and degradation mechanism of photo-degradation of BPA using a photo-reactor system with UV. We examined the effects of initial BPA concentration, UV intensity, pH, and nitrate concentrations on the photolysis of BPA in water. After irradiation of 4 hr, photo-removal efficiency of BPA approached to 90% using UVC light whereas BPA was hardly removed with UVA light. The pseudo-first-order rate constants of BPA photolysis with UVC decreased from  $8.5 \times 10^{-3}$  to  $3.7 \times 10^{-3} \text{ min}^{-1}$  with increasing initial concentration of BPA from 2 to 40  $\text{mg L}^{-1}$ . The pseudo-first-order rate constants of BPA increased from  $5.74 \times 10^{-3}$  to  $14.94 \times 10^{-3} \text{ min}^{-1}$  with increasing UV intensity from  $3.65 \times 10^{-5}$  to  $9.68 \times 10^{-5} \text{ einstein L}^{-1} \text{ min}^{-1}$ . The quantum yield of BPA was found to be  $0.0364 \text{ mol einstein}^{-1}$  in UV photolysis. Photodegradation of BPA was significantly improved by increasing pH close to  $\text{pK}_a$  value of BPA (10.2). The degradation rate of BPA was also enhanced with increasing nitrate concentration, up to 40  $\text{mg L}^{-1}$  for BPA, and then decreased. This result indicates that OH and  $\text{NO}_2$  radicals produced by nitrate ion might play important roles in photolysis of BPA, but at higher nitrate concentration, nitrate ion might act as a radical inhibitor. The intermediates during photolysis of BPA were identified by using LC/MS/MS in the absence and presence of nitrate ion. 4-hydroxybenzaldehyde, 1-(4-hydroxyphenyl)-ethanone, 2-(4-hydroxyphenyl)-2-methylpropanal, and 4-(2-(4-hydroxyphenyl)propan-2-yl)benzene-1,2-diol were found in both reactions, however, nitro-intermediates such as 4-nitrophenol, 4-(2-nitropropan-2-yl)phenol, 4-(2-(4-hydroxy-3-nitrophenyl)propan-2-yl)-phenol, and 4-(2-(4-hydroxy-3-nitrophenyl)propan-2-yl)-benzene-1,2-diol during photodegradation of BPA in the presence of nitrate ions were additionally produced. These intermediates were degraded with further reactions. Our results can be helpful to understand the kinetics and removal mechanism of photo-degradation of BPA in the treatment process and in natural water environment, especially in the presence of common nitrate ions in water.

**RP097 Sediment-associated steroids and their bioavailability to fish: Evaluating appropriate methodologies** A.M. Jessick, University of Nebraska Medical Center/ Univ. of Neb. / Department of Biology, University of Nebraska Medical Center/ Univ. of Neb. – Omaha / Nebraska Watershed Network, Dept. of Biology, University of Nebraska Medical Center/ Univ. of Neb. – Omaha / graduate research assistant; L. Knight, A. Kolok, University of Nebraska at Omaha / Nebraska Watershed Network, Dept. of Biology. Trenbolone, an anthropogenic chemical growth promoter used in cattle, has been detected in water and sediment at  $\mu\text{g}/\text{L}$  concentrations. Studies have shown sediment-associated trenbolone is bioavailable to aquatic organisms leading to reductions in hepatic gene expression of vitellogenin, but few studies have investigated the route by which sediment-associated trenbolone becomes bioavailable to fish. The objective of this study was to determine the route of exposure (ventilation or ingestion) of trenbolone associated with sediment to the fathead minnow (*Pimephales promelas*) utilizing two methods: a ligation procedure and a double tank system. In the ligation method,

preliminary studies examining the effect of starvation and surgical stress were conducted on female fathead minnows. No significant differences were observed in the hepatic expression of vitellogenin in female minnows after a 14 d fast, nor were their significant differences in vitellogenin expression due to surgical stress. Results from the ligation study were mixed, while the ligation procedure appeared to be effective during a 7 d exposure, the procedure was not viable after 14 d. As such, an alternative method was devised to investigate the route by which sediment-associated trenbolone becomes available to fathead minnows, featuring a double tank system. For this study, a negative control containing lab water, a positive control ( $5 \mu\text{g}/\text{L}$  trenbolone) and an experimental treatment were examined after 14 days. The experimental tank consisted of a 15 L tank containing a stainless steel mesh lid placed inside a 90 L tank, followed by the addition of trenbolone spiked sand and overlain with water. Ten female fathead minnows were placed inside the inner 15 L tank and ten females were located outside the 15 L tank. No significant differences were observed between the fish in the inside tank and outside tank of the experimental treatment. However, the two experimental groups were significantly different from the negative control. Despite the fact that a small amount of particulate material entered the inner tank, these results suggest that the major route of exposure of trenbolone to the fathead minnow is ventilation, rather than sediment ingestion. Additional studies with different matrices, organic matter, and particle size are needed to better understand the route of exposure and bioavailability of steroidogenic compounds to aquatic organisms.

**RP098 Temporal Distribution, Risk Assessment and Fluxes of Endocrine Disrupting Chemicals in the Surface Water of the Pearl River System, South China** J. Gong, Guangzhou Institute of Geochemistry / State Key Laboratory of Organic Geochemistry; Y. Ran, Guangzhou Institute of Geochemistry / Chinese Academy of Sciences; Y. Yang, Guangzhou Institute of Geochemistry / State Key Laboratory of Organic Geochemistry. The pollution levels of selected endocrine disrupting chemicals (EDCs) were seasonally investigated in riverine surface water of the Pearl River system, South China. The aqueous concentrations ( $\text{ng}/\text{L}$ ) of 4-*tert*-octylphenol (OP), 4-nonylphenol (NP), bisphenol A (BPA), estrone (E1) and 17 $\beta$ -estradiol (E2) were in the ranges of  $< 1.6$ –577, 276–14936, 8.7–639.1,  $< 1.5$ –17.6 and  $< 1.1$ –1.7, respectively. In general, the concentrations of EDCs displayed a seaward decreasing tendency, and their distributions were mainly related to the anthropogenic emissions along the rivers. Seasonally the trend that the EDC concentrations in the Zhujiang River (ZR) and Dongjiang River (DR) are generally higher in summer than in winter was observed. Moreover, the positive correlation between EDC concentrations and dissolved organic carbon (DOC) contents was also found excluding the point-source sites. In a worldwide comparison, the surface water of the Pearl River system have been seriously contaminated by the phenolic xenoestrogens (OP, NP and BPA), while the pollution of natural estrogens (E1 and E2) was at low level. However, it was noteworthy that the estrogen equivalent (EEQ) values of the investigated surface waters of the Pearl River system have mostly reached or exceeded the reported estrogenic effect thresholds, indicating the threat of EDCs to the local aquatic species is expected, and especially high-ecological risks have been observed in the ZR and DR. In addition, it was roughly estimated that approximate 315.7 and 56 metric tons/yr of alkylphenols (APs) and BPA were inputted into the coastal ocean from the Pearl River Delta, respectively, through the riverine runoff.

**RP099 The Analysis of Surface Waters for Targeted and Non-Targeted Analyte Identification and Quantitation by Accurate Mass LC/MS/MS** P. Winkler, C. Borton, AB SCIEX. The introduction of fast, high resolution Quadrupole Time of Flight mass spectrometers has provided the environmental testing community with a powerful tool for the identification and quantitation of compounds in water samples. Data from the analysis of a variety of surface waters will be presented that will demonstrate the importance and utility of fast data acquisition, high resolution MS/MS spectra and intuitive data processing to identify compounds present in the samples without any prior knowledge of their content. The general unknown screening (GUS) workflow will be presented along with a discussion of important considerations for its proper use. The process for developing a quantitative analytical method for the identified unknown compounds in the GUS phase of sample investigation will also be presented.



**RP100 Understanding sucralose in the North Bosque River, Texas, USA using SWAT: Measured and predicted concentrations in an effluent-dominated watershed** C.S. Breed, B. Du, Department of Environmental Science, Center for Reservoir and Aquatic Systems Research, Baylor University; K.C. Chambliss, Department of Environmental Science, Center for Reservoir and Aquatic Systems Research, Baylor University / Department of Environmental Science, Center for Reservoir and Aquatic Systems Research; J.G. Arnold, Grassland Soil and Water Research Laboratory, U.S. Department of Agriculture; B.W. Byars, Center for Spatial Research, Baylor University; B.W. Brooks, Department of Environmental Science, Center for Reservoir and Aquatic Systems Research, Baylor University. On ongoing effort by U.S. Department of Agriculture includes modifying the Soil Water Assessment Tool (SWAT) to understand watershed fate and transport of contaminants of emerging concern (CECs). SWAT was used in this initial study with targeted CECs for the North Bosque River basin of central Texas, USA, with monthly precipitation during 2011 and 2012. The purpose of this project is to assess transport of selected CECs discharged from wastewater treatment plants in the North Bosque River basin under various instream flows. Sampling events included an extreme drought during summer months of 2011 when instream flows of the North Bosque River were largely dependent on effluent discharges. We initiated our work with sucralose, because it has been previously identified as a conservative tracer of municipal effluent. Sucralose and other CECs (e.g. pharmaceuticals, caffeine) were quantitated using LC/MSMS with isotope dilution from 9 sampling stations during multiple sampling events. Sucralose concentrations ranged from 1-9 ug/L, whereas concentrations of other CECs were general consistent with previous reports in the literature from the USA. The pesticide function in SWAT was used to insert various physicochemical properties and concentrations of sucralose and other CECs. Our findings compare field observations with model outputs of sucralose, and highlight the opportunities and present challenges associated with using SWAT for CEC modeling at the watershed scale.

**RP101 Uptake and depuration of the pharmaceuticals in reclaimed water by mosquito fish (*Gambusia holbrooki*)** J. Wang, Florida International University / Chemistry & Biochemistry, SERC; P. Gardinali, Florida International University / Department of Chemistry and Biochemistry. The reclaimed water used for the ground irrigation at Florida International University Biscayne Bay Campus (North Miami Beach, FL) was previously analyzed and 29 pharmaceuticals including analgesics, antidepressants, antibiotics, antihistamines, antihyperlipidemics, antifungals, beta blockers were consistently detected and reported. Because these pharmaceuticals are continuously released to a freshwater pond through daily irrigation, our working hypothesis is that they will bioaccumulate in the mosquito fish (*Gambusia holbrooki*) living in this pond. Initial analysis of the native fish showed the presence of a group of the compounds in the reclaimed water at various concentrations. In order to understand the pharmacokinetics and potential hazards of these pharmaceuticals and some of their metabolites, 60 mosquito fish were exposed to reclaimed water in the lab under static conditions for 7 days with daily 50% renewal of the reclaimed water. 3-5 fish were sampled on 5h, 1d, 2d, 3d, 5d and 7d. 280 mL of the water were sampled at the time of fish sampling or on a daily basis, whichever is more frequent. After the 7-day uptake phase, the fish were transferred to another tank filled with clean water and allowed to depurate for 21 days with daily renewal of the water. 3-5 fish were sampled on 3, 7, 10, 14, 21d. 500 mL of the water were sampled from the tank at the time of fish sampling or on a daily basis, whichever is more frequent. 30 mosquito fish were maintained in a separate tank filled with clean water as a control group. 10 mosquito fish were collected on 0 and 7d of the uptake phase and 21d of the depuration phase. 9 pharmaceuticals including trimethoprim, caffeine, metoprolol, diphenhydramine, carbamazepine, erythromycin, fluoxetine, bezafibrate and diclofenac were detected in mosquito fish. The calculated bioconcentration factors were 6.1, 8.1, 25, 145, 3.4, 81, 1171, 395 and 137 respectively.

**RP102 Utilization of GCxGC-TOFMS as a Broad-Spectrum Analysis for Endocrine Disruptor Compounds in Urban and Rural Watersheds** J. Patrick, J. Binkley, J. Heim, LECO Corporation. New classes of compounds are being introduced into the ecosystems every day. These compounds are often later determined to have deleterious effects on the ecosystem. One of these groups is referred to as the Endocrine Disruptor Compounds (EDCs) which are a diverse collection of chemicals, including drugs, pesticides, polymer additives, personal consumer products, industrial

by-products, and pollutants. There is worldwide concern over long-term environmental exposure to EDCs leading to serious health effects including a range of reproductive problems such as reduced fertility, male and female reproductive abnormalities, skewed male/female sex ratios, brain and behavioral problems, impaired immune functions, and various cancers. This research presents a robust, broad range analysis for the detection of EDCs in impacted natural waters using Comprehensive Two-dimensional Gas Chromatography–Time-of-Flight Mass Spectrometry (GCxGC-TOFMS). GCxGC facilitates enhanced detection, chromatographic resolution, and peak capacity while TOFMS allows the fast acquisition (up to 500 spectra per second) necessary to successfully acquire the data density needed to fully characterize low levels of targeted and untargeted compounds in complex samples. Comprehensive two-dimensional gas chromatography coupled with time-of-flight mass spectrometry was utilized in this research to detect endocrine disruptors, personal care products, and other pollutants in samples obtained from urban and rural point sources along a Midwestern watershed. A solid phase extraction method was developed using a hydrophilic modified styrene-based polymer to capture a broad range of compounds from aqueous samples. An optimized GCxGC method was developed using a conventional non-polar and mid-polarity column set with variable modulation to optimize peak capacity and chromatographic resolution. TOFMS utilizing True Signal Deconvolution® and fast acquisition rates was utilized for detection. The analysis of EDCs detected 102 chemicals in aqueous extractions from 6 different point sources along the watershed. The detected compounds matched the reference standard with at least a 60% library match similarity. Furthermore, results show that 81% of the 102 chemicals detected were found at least 5 times. GCxGC-TOFMS provides an excellent tool for the untargeted detection of pollutants in waterways. The data presented illustrates the advantages and benefits of GCxGC-TOFMS.

**RP103 Endocrine Disruption and Hepatic Protein Expression in Contaminant-Exposed English Sole in the Southern California Bight** C.M. Waggoner, California State University, Long Beach / Environmental Endocrinology Laboratory; J.A. Reyes, Pacific Coast Environmental Conservancy; J.L. Armstrong, Orange County Sanitation District; B.J. Allen, California State University, Long Beach; K. Kelley, California State University, Long Beach / Environmental Endocrinology Laboratory, California State Univ, Long Beach. In urban-impacted coastal waters of southern California, we have measured a variety of environmental contaminants that accumulate in the tissues of resident English sole (*Pleuronectes vetulus*). In studies of this species, and others, exposures to contaminants are increasingly being tied to important, potentially deleterious, phenotypic changes. These include altered endocrine regulatory pathways, detoxification responses, and larger physiological systemic effects (e.g., on metabolism, growth, reproduction). Findings also indicate that different types of phenotypic effects are significantly correlated with exposures to distinct classes of contaminants. For example, increasing tissue concentrations of chlordanes are significantly related to thyroid disruption, while certain polychlorinated biphenyl (PCB) congeners and biphenyl were instead significantly related to disruption of the stress-response (cortisol) endocrine system. Using 2D gel electrophoresis and MALDI-TOF-TOF mass spectrometry to identify and measure protein expression profiles in tissues of impacted fish, our research is discovering previously undocumented phenotypic effects. Protein expression in liver indicate a number of changes in detoxification processes (e.g., in GST, Se-binding protein), metabolic adaptation (e.g., catabolic enzyme changes), cellular acclimation (e.g., HSPs, signaling, cell structure), and oxidative stress (e.g., catalase, peroxiredoxin), among others. These changes appear to be related to different types of endocrine disruption and contaminant exposures. An integrative analytical approach, based on multiple measures, is beginning to point to underlying mechanisms of environmental impacts and their potential causative agents. (Supported by NOAA-USC Sea Grant Program and CSU COAST).

**RP104 The Case for Case Studies: The UNEP-SETAC Life-cycle Initiative Phase 3** B.W. Vigon, SETAC; S. Valdivia, S. Lemmet, United Nations Environment Programme; J. Fava, Five Winds International; G. Sonnemann, University of Bordeaux. The UNEP-SETAC Life Cycle Initiative is completing its tenth year of existence and bringing to a close Phase 2. The past five years saw major accomplishments in the form of guidance, workshops, LCA and LCM practices and tools development and growth of the network. This presentation will highlight a number of these accomplishments. More to the point, support from stakeholders and affirmation from

the International Life Cycle Board has led to the development of a strategy and plan for a Phase 3, which will start in January 2013 and run through 2017. Key program areas and flagship projects will be overviewed. The SETAC North America meeting will serve as the North American launch of Phase 3. One of the areas of interest for the Phase 3 effort is how best to mainstream life cycle approaches. Case studies have been suggested as one component of mainstreaming, particularly as a way to validate methods and to capacity build. This presentation will present some arguments pro and con and illustrate some instances where benefits of case studies are significant.

**RP106 Normalization and Weighting in Comparative LCA- Transportation Fuels Case Study** V. Prado, K. Soratana, A. Landis, Arizona State University / School of Sustainable Engineering and The Built Environment; S. Tylock, Sustainable Intelligence; T. Seager, Arizona State University / School of Sustainable Engineering and The Built Environment. Comparative life cycle assessment (LCA) evaluates the relative performance of multiple products, services, or technologies with the purpose of identifying the least impactful alternative. Current ISO standards for performing normalization and weighting are optional, insufficient and generally lead to an oversimplified biased answer. Therefore, most LCA studies provide only characterized data, leaving decision-makers subject to systematic biases when assessing tradeoffs. This study applies a novel normalization and weighting scheme to a comparative LCA of transportation fuels. The novel approach consists of internal normalization using outranking, and application of probabilistic weights, following a general process called Stochastic Multi Attribute Analysis (SMAA). While traditional methods yield results that are highly aggregated, the SMAA method generates a result in terms of the probability distribution of the overall scores, and displays multiple viewpoints from decision makers. Transportation fuel represents an exceptional case study because of the high level of tradeoffs between alternatives (i.e. global warming vs. eutrophication). In all, SMAA may be used to better guide the decision making process, handle uncertainty, and avoid making decisions that trade one environmental impact for another.

**RP108 The Extension of Life-Cycle Assessment to Dredging and Sediment Disposal Strategies** M.E. Bates, US Army Corps of Engineers / Environmental Lab, Engineer Research and Development Center; B.A. Wender, Arizona State University; L. Seymour, Carnegie Mellon University; K. Kelly, Massachusetts Institute of Technology; T.S. Bridges, I. Linkov, US Army Corps of Engineers, Engineer Research and Development Center. Managing contaminated sediments in a way that properly balances environmental risks and benefits is often a point of controversy between federal agencies and with the public. There are a number of sediment disposal strategies, including landfilling, deep-sea dumping, confined disposal, and beneficial reuse (e.g., wetland restoration, cement manufacturing), each with differing life-cycle profiles. While public opinion often favors placing even minimally contaminated materials in a landfill, the environmental impacts associated with the additional moving and handling activities of this preference may outweigh impacts originating from the contamination itself. Specifically, a variety of distributed and long-term impacts can result from transportation by truck or barge, use of loading equipment, and long-term management, with implications for carbon emissions, environmental and occupational health, and social welfare. Long-term and distributed environmental benefits from beneficial uses of dredged sediments are also not quantitatively considered in current planning decisions. Life-Cycle Assessment (LCA) accounts for a more complete range of impacts and benefits than do existing risk assessments of sediment-management strategies. For example, the use of LCA in the related field of soil remediation has shown that risks originating from remediation processes often exceed the environmental risks from the prior contamination; by developing an LCA for dredging operations, these pitfalls can be avoided for future sediment-management decisions. This presentation extends LCA to dredging and sediment management by building on traditional LCA concepts through additional life-cycle data collection, development of inventories of life cycle impacts caused by common dredging and sediment placement activities, and development of impact assessments for these operations. These new methods for LCA are applied in a comparative analysis of operational dredging and disposal strategies in a case study of the Long Island Sound region of NY/CT.

**RP109 Opening the Gate: End of Life Impacts on the Life Cycle of Biopolymers** T.A. Hottle, A.E. Landis, Arizona State University; M.M. Bilec, University of Pittsburgh. Bio-based materials are being produced to displace petroleum-based materials with the goal of creating more environmentally friendly products. Biopolymers, materials derived from biological feedstocks and used in the production of plastics, are being produced with the added features of compostability and biodegradability, but it is not clear what benefits, if any, these methods of disposal may have over traditional waste management strategies. A review of biopolymer life cycle analyses demonstrates the need to expand biopolymer research into the use phase and end of life scenarios to explore these issues. The use phase determines the length of time a plastic remains in circulation and which types of polymers are being produced. The use of biodegradable and recyclable polymers defines which end-of-life scenarios are possible for the polymers, broadening the available management strategies. At the end-of-life the polymers are met with several possible scenarios, each resulting in different outcomes in regards to carbon output and co-products. These scenarios result in different impacts which alter the life cycle of biopolymers, they include recycling, landfilling, composting, and incinerating, yet many life cycle analyses of biopolymers do not include end of life in the system boundaries. The end-of-life management of biopolymers influences how the carbon embedded within the plastics is reintroduced into the atmosphere, if at all, in addition to other significant environmental flows, and is largely dictated by the end of life scenario. This poster presents the carbon footprints for the end-of-life scenarios for two common biopolymers, polyhydroxybutyrate and polylactic acid.

**RP110 Assessing the Sustainability of Recycling Programs in Healthcare** S. Unger, Arizona State University / School of Sustainable Engineering and The Built Environment. The growth and evolution of the US healthcare system has correlated with its impact on the environment; with the growing amount of single use products used in healthcare comes an increase in medical waste. This rise in medical waste has several consequences, such as the increase in needed materials, or, a higher potential for secondary contamination resulting from the waste, as more waste must be handled and/or disposed. To compensate, there are several programs worldwide that specifically focus on the recycling of medical waste. However, there exists minimal literature regarding the sustainability of these medical waste recycling programs and what may be their attributed environmental impacts. This poster presents several different options for handling and/or reducing medical waste and their associated environmental impacts. The sustainability of medical waste recycling programs will be evaluated from a life cycle perspective.

**RP111 Life Cycle Assessment of Anesthetic Agents** C. Le, Yale University; J. Sherman, Yale School of Medicine; V. Lamers, Yale University; M. Eckelman, Northeastern University. The present study uses life cycle assessment (LCA) to examine the climate change impacts of five anesthetic drugs, sevoflurane, desflurane, isoflurane, nitrous oxide and propofol. A full cradle-to-grave approach was utilized, encompassing resource extraction and manufacturing of the anesthetic agents, transport to healthcare facilities, drug delivery, and disposal or emission to the environment. Along each stage of the life cycle, energy and material inputs and emissions are considered, and the use phase considers technology and energy requirements specific to the delivery of each agent. Desflurane accounts for the largest life cycle greenhouse gases (GHGs) impacts among the anesthetic drugs considered: 15 times that of sevoflurane and 20 times that of isoflurane when O<sub>2</sub>/air is used, with GHG emissions increasing significantly for all agents when N<sub>2</sub>O/O<sub>2</sub> is used. For all of the inhaled anesthetics, GHG impact is dominated by uncontrolled emissions of waste anesthetic gases. GHG impacts of propofol are comparatively quite small, nearly four orders of magnitude lower than those of desflurane or nitrous oxide. The GHG impacts of propofol primarily stem from the energy needed to operate the syringe pump and not drug production or direct release to the environment. Our results reiterate previous published data on the GHG effects of these inhaled agents in the context of a full life cycle. Several practical environmental impact mitigation strategies exist including the restriction of desflurane and nitrous oxide unless they would reduce morbidity and mortality over other agents. Strong consideration should be given to the employment of waste anesthetic gas capturing systems currently in existence. From our results, it appears that techniques, such as TIVA, neuraxial, or peripheral nerve blocks, may be least harmful to the environment.

**RP112 Addressing Tradeoffs in Diet Variability for Life Cycle Assessment: A Case Study** A.J. Berardy, Arizona State University / School of Sustainability; T.P. Seager, Arizona State University / School of Sustainable Engineering and The Built Environment. Typical dietary life cycle assessments (LCA) dealing with plant-based diets make the assumption that those following a vegan diet do not replace animal products with processed alternatives. This oversimplification obscures the results of the assessment, making it invalid for those not following the diet described in the LCA. This can act as a barrier to sustainable consumption decisions when the retail market is already saturated with competing environmental claims frequently made without empirical data, green-washing, and use of unregulated terms like “natural”. Other considerations including cost, health and ethical concerns make it even harder to make the right choice. Advocates of plant-based diets argue that eating vegan foods will not only benefit animals, but also be better for the environment, and dietary LCAs support this. In theory, eating plants is likely to have a lower environmental impact than eating animals, in so far as eating at a lower trophic level requires less energy input for the same nutrition. However, skeptics might counter that the LCAs do not accurately reflect the typical vegan diet, and including processed alternatives would increase the overall impact of that diet. The wide variety of vegan processed alternatives to animal products including cheese, beef, milk and eggs have not been examined using LCA even though for many vegans this constitutes a significant portion of their diet. Some of these products are more demanding of resources due to their extensive industrial processing and use of artificial ingredients. This raises the question of whether some vegan products may be more impactful than their animal equivalents. To start answering this question, a consequential LCA of seitan and beef is presented. This comparative LCA also provides a pathway to further reducing environmental impacts from diet and a way to quantify exactly what benefits are gained through this sustainable consumption decision.

**RP113 A case for coupling evaporatively cooled air conditioning and decentralized greywater treatment in the residential sector** E.R. McKenzie, University of California Davis / Civil and Environmental Engineering; T.E. Pistochini, University of California Davis / Western Cooling Efficiency Center; F.J. Loge, University of California Davis / Civil and Environmental Engineering; M. Modera, University of California Davis / Western Cooling Efficiency Center. Total energy and water burdens, including both direct and indirect uses, were modeled for newly constructed and 15-year-old homes in five California climate zones for three cooling systems: air-cooled, air-cooled with evaporative pre-cooling, and evaporative condenser air conditioners (A/C). The total energy burden of evaporative condenser air conditioners was less than that of air-cooled with evaporative pre-cooling, which was less than that of air-cooled A/C. Evaporative condenser A/C use yielded substantial energy savings (11% average, 37% maximum) compared to conventional air-cooled A/C. In hot and arid environments, evaporative condensers offer a promising approach to reducing peak energy demand, as A/C use is typically associated with peak demand and evaporative condenser energy savings compared to air-cooled AC increase with increased temperature. Air-cooled unit had a lower total water burden than the two evaporative technologies, which exhibited similar water burdens. Direct water use by the evaporative technologies amounted to 2-8% addition water use annually for a typical California house. This additional water burden can be offset by implementing an in-home decentralized graywater treatment and reuse plan, where shower and clothes wash water is treated and recycled in-home for evaporative cooling as well as other beneficial re-uses, such as irrigation and toilet flushing. Treated graywater can eliminate the additional potable (tap) water burden, thus giving evaporative condensers a lower total energy and water burden than that of conventional air-cooled units. Finding an alternative water source is particularly important in regions where more air condition is needed (i.e., more full-load hours) or where local water quality indicates high bleed rates to improve long-term performance; treated graywater offers a potential solution.

**RP115 LCA of engineered nanomaterials, case study of a Mexican mining company** E. Alvarado-Díaz, CIMAV / Environmental Science and Technology, CIMAV / Environmental Science and Technology; G. Gonzalez Sanchez, Centro de Investigación en Materiales Avanzados (CIMAV) / Department of Environmental Science and Technology; M. Miseljic, S.I. Olsen, Technical University of Denmark / Management Engineering, Quantitative Sustainability Assessment; J. Bocanegra, Servicios Industriales Peñoles S.A. DE C.V.. The application and study of nanomaterials have

drawn increasing attention due to their unique and novel properties being used in the development of new products and technologies. However important uncertainties and knowledge gaps remain in the field of engineered nanomaterials (ENMs) regarding to their potential repercussions in human health and environment. Such effects are difficult to quantify owing to the low understanding of their potential fate and exposure rates in ecosystems. Currently, Life Cycle Assessment (LCA) is a suitable method that can be applied to quantify the environmental impacts of products or systems within established boundaries (e.g. cradle-to-grave or cradle-to-gate) and potentially compare it to other product alternatives. Additionally, it is a valuable tool in the innovation of products. This case study considers the cradle-to-gate production of a Zinc Oxide nanoconcentrate (ZnO NCE) and its incorporation in a polypropylene (PP) matrix, producing PP pellets with UV protection properties. UV light protection is an important feature against the photodegradation. These ZnO PP based nanocomposites (NC) have a wide range of applications since they represent a novel additive technology for the plastic industry. The purpose of this study was to evaluate, by LCA methods and software simulation, the potential environmental impacts of the ZnO PP based NC manufacturing at a Mexican production site. The data collected and the conditions represented were aimed toward the geographical scope of Mexico. Data on production of ENMs was not easily attainable and, to a certain extent, the use of generic processes was required. The results indicate that the data used for this LCA are at one point uncertain, as it is difficult to achieve complete data sets that make a good inventory of the process. The outcomes show that through the PP production process occur the highest impacts associated to the final product. Regard to the ZnO NCE synthesis, hotspots for potential improvements were found in the phase of ZnSO<sub>4</sub> extraction, however, the overall impacts of the synthesis are not high, due they applied green chemistry principles. The LCA results can also be used to determine if the production of ZnO PP based NC has a better environmental performance than the present applied additives technologies, which the ENMs can replace. However, at this stage, it has not yet able to include impacts from the potential release of ENMs to the environment.

**RP116 Life-Cycle Assessment of Natural Fibre-Reinforced Composites From South Eastern Nigeria** I.N. Onwurah, University of Nigeria / Dept of Biochemistry, University of Nigeria, Nsukka / Department of Biochemistry, University of Nigeria / Biochemistry, University of Nigeria; A.C. Victor, University of Nigeria Nsukka / Department of Biochemistry. In our previous work using LCA methodology, it was shown that the cost-effectiveness of the microbial degradative pre-treatment method of lignocellulose is highly glaring, both in terms of material use and global warming. Thus it was highly recommend that industries imbibe this method for their pre-treatment of such material. Consequent upon above, we considered it essential to adapt LCA methodology to compare, in terms of composite manufacture, distribution, disposal and/or re-use, our tropical biofibres (product) quality derivable from *Ampelocissus leonensis*, *Ampelocissus cavicaulis*, *Adenia lobata*, *Cissus palmitifida*, *Morinda morindoides*, *Gongronema latifolia*, and *Urena lobata* of tropical forests of South Eastern Nigeria with those made from conventional ones, so as to identify the major contributions and impact indicators from raw materials acquisition, processing and characterization. From economic, pollution and ecological point of view, the use of plant fibres is favourable because of their carbon (iv) oxide neutrality, biodegradability, sustainability and cleaner processing conditions and also is often associated with an eco-design initiative for introducing environmentally friendly materials. By referring to some life-cycle inventory, it was shown that fibre quality is largely dependent on the retting condition and duration. We also found that the natural water retting condition, an indicator relevant to the LCA model, offers the best extraction method for fibres. Water retting process utilised the least energy but generated a considerable amount of waste water. The moderate Kurschner Hoffer cellulose contents and relatively high klason lignin contents of the fibres from these tropical plants were indications of high mechanical strength. The cellulose with high lignin content is also an indicator of stiffness and low pulp yield. Thus, such fibres would be suitable for composite reinforcement.

**RP117 A LCA on MCQ-Treated Lumber Compared to ACQ-Treated Lumber** M.P. Tsang, Association of Schools for Public Health Fellow hosted by the U.S. EPA; D.E. Meyer, W.W. Ingwersen, T. Hawkins, U.S. EPA / Sustainable Technology Division, Systems Analysis Branch. This project demonstrates how a cradle-to-grave life cycle assessment (LCA) can



be applied to the assessment of a nano-product. ISO 14040 and 14044 have been designated as appropriate methods for conducting LCAs on nanomaterials. A lack of data and a lack of specificity in impact assessment methodologies to accurately assess nanomaterials have stifled much meaningful use in this manner. However, given the pace at which nano-products are being introduced into commerce, quantitative assessments are needed now even if impacts might be uncertain. This LCA compares two raised-garden beds: one treated with soluble copper as alkaline copper quaternary (ACQ) and the other treated with copper nanoparticles as micronized copper quaternary (MCQ). The voluntary US phase-out of chromium copper arsenate (CCA) in 2003 has led to the use of copper nanoparticles at more than 70% of the treated wood market in North America. MCQ formulations contain copper particles 90% of which are < 1,000 nm and generally in the range of 10 nm to 700 nm. The environmental and public health consequences of this transition in the wood treatment industry are still not understood. Results of this assessment are used to examine the trade-offs associated with using nano-treated lumber alternatives to conventional lumber products. Although current LCA methods lack characterization factors that accurately model human health effects and ecotoxicology of nanomaterials, a qualitative assessment highlights critical spots along the life-cycle to demonstrate where further research and development can enhance LCA as a tool for making informative assessments on ENMs. The functional unit of this LCA is described as both a raised-garden bed of 16 square feet (ft<sup>2</sup>) per year of use and as 1,000 board feet (Mbf). Raised-garden beds provide a unique high-exposure scenario that may have a salient effect on the toxicity impacts. A complete cradle-to-grave model and life-cycle inventory is built for nanoscale copper carbonate for the first time that serves as basis for future LCA work in this field. Mid-point impacts are calculated using the EPA's Tool for the Reduction and Assessment of Chemical Impacts (TRACI, v.3.0) for: global warming (kg CO<sub>2</sub> eq), acidification (H<sup>+</sup> moles eq), eutrophication (kg N eq), ozone depletion (kg CFC-11 eq), smog (kg NO<sub>x</sub> eq), carcinogenics (kg benzene eq), non-carcinogenics (kg toluene eq), respiratory effects (kg PM<sub>2.5</sub> eq), and ecotoxicity (kg 2,4-D eq).

**RP119 A Case Study in Identifying Safer Alternatives – Traffic Paints** B. Penttilä, Pacific Northwest Pollution Prevention Resource Center. Tens of millions of gallons of traffic paint are used each year by governments and private businesses to mark highways and parking lots across the United States. For decades, lead-based pigments were the colorant of choice in the most common yellow traffic paints. Due to the toxicity of lead-containing pigments, new non-lead pigments for paints and non-paint products have been introduced for traffic marking. This work examines the comparative hazards of traffic paint formulations with and without lead-based pigments using two methods, the GreenScreen for Safer Chemicals (developed by Clean Production Action) and the Quick Chemical Assessment Tool (developed by the Washington State Department of Ecology). Uses of these formal methodologies reveal hazard considerations that may be overlooked in more casual ad hoc product safety comparisons. Similarly, successful substitution of hazardous chemicals with safer alternatives requires a thorough analysis of the functional use of product constituents and a full consideration of the way in which changes in individual components can affect seemingly unrelated but important product attributes. Characteristics of paint formulations with inorganic pigments and other purportedly “safer” formulations with organic pigments will be compared. These results will be extended to consider the influence of pigments and other components for a broader range of paint products. The principle route of environmental exposure to lead pigments in traffic paints is stormwater runoff. Recent policy analyses suggest that end-of-pipe monitoring and control are not likely to adequately control stormwater's contribution to water body impairment. This suggests the need for policies that drive reduction in content of hazardous chemicals and that incentivize the development of safer alternative formulations for traffic paints.

**RP120 Application of LCA to Consumer Product Environmental Claims** C.F Isaacs, Contemporary Information Analysis Ltd.. An increasing number of consumer products carry environmental claims of one kind or another but consumers and critics continue to report that they are not sure that they can trust these claims. The international standard ISO 14021, which provides guidance on self-declared environmental claims, does not require a full Life Cycle Analysis to be carried out to verify an environmental claim but it does require consideration of the life cycle of the product for which the claim is being made. Manufacturers and brandowners face many

key challenges when attempting to conduct an LCA for a typical consumer product. Issues include selection of appropriate products with which to compare the greener product, variability of manufacturing processes, transportation from manufacturer to warehouse to retail store, and the variability of recycling and composting options accessible to consumers. Government programs that focus on a single attribute, for example waste reduction, while ignoring other attributes, can also add complexity to the definition of a greener product. Few manufacturers want to face the cost of a full LCA but use of Life Cycle Inventory databases may lead to misleading claims. This presentation is based on analysis of the results of more than 20 years of independent verification of environmental claims for one of Canada's largest brandowners and will be illustrated with case studies including bottled water, shopping bags, food packaging, and household cleaning products.

**RP121 Erosion analysis Related to Corn-based Ethanol production in the US** J.C Bare, US EPA / MS-466, US EPA. Since the Renewable Fuel Standard has encouraged the development of biofuels, the US has seen an increase in corn production for conversion to ethanol. In many of these agricultural regions, increased corn production is accompanied with increased erosion. An erosion analysis will be presented that includes one regional scenario analysis of 12 US Midwestern states which is responsible for 88% of the corn production. Additional modeling will be done in the future to expand this analysis projecting two possible future scenarios for land use in 2020. The ArcGIS modeling was conducted with a 30 meter x 30 meter grid defining land use / land cover. The Natural Capital's InVEST was used to provide additional insights into erosion at each 30 meter x 30 meter location.

**RP122 Overview of Monitoring Conducted in Support of the Los Angeles and Long Beach Harbors Toxics TMDL** A. Jirik, K. Curtis, Port of Los Angeles; M. Arms, Port of Long Beach; A. Crumpacker, D. Moore, Weston Solutions, Inc. The recently adopted Los Angeles and Long Beach Harbors Toxics Total Maximum Daily Load (TMDL) requires that special studies, including harbor-specific sediment and fish tissue linkage studies, will be developed and implemented within the Harbor Complex. These studies are crucial to determine allowable sediment contamination levels in the Harbor Complex which will result in compliance with fish tissue numeric targets. Compliance with the TMDL will likely be based on sediment quality objectives (SQOs), which were adopted by the California State Water Resources Control Board in 2009 to assess the direct effects of pollution in sediments on benthic communities. The direct effect SQOs use a multiple lines of evidence approach and include sediment chemistry, sediment toxicity, and benthic community assessments. Currently, Phase 2 of the SQOs is under development, and focuses on the indirect effects of sediment contamination. Critical to TMDL implementation is that the SQO Phase 2 decision support tool focuses on prediction of the proportion of fish tissue contamination from sediment pollution. Within the Harbor Complex, it is not currently known how much of the observed fish tissue contamination can be linked to local contaminated sediments. This makes compliance with the TMDL, which requires clean-up of contaminated sediments to levels which will result in fish tissue numeric target compliance, difficult. The Ports of Los Angeles and Long Beach have devoted significant resources to the development of a hydrodynamic, water quality and sediment transport model, fish tracking studies, and sediment collection, analysis and reporting in support of the TMDL. In 2011, fish tissue, sediment, and overlying water samples were collected for the purpose of developing a sediment and fish tissue linkage analysis. This dataset represents the first major effort in the State focused on collecting data for the purpose of developing sediment and fish tissue linkage. This presentation focuses on initial linkage evaluations between sediments and observed fish tissue concentrations as well as a comparison of the 2011 results to Palos Verdes shelf studies. Final results of these analyses will be important for the development of the TMDL monitoring and implementation plans, as well as to calculate revised sediment numeric targets.

**RP123 A proposal for Considering Mixture Toxicity with EQS Compliance Checking** M. Junghans, Swiss Centre for Applied Ecotoxicology EAWAG – EPF / Ecotox Centre, Swiss Centre for Applied Ecotoxicology EAWAG – EPFL; P. Kunz, Swiss Centre for Applied Ecotoxicology EAWAG – EPFL; M. Vighi, University of Milano Bicocca / Department of Environmental Sciences, University of Milano / Department of Environmental Sciences; L. Werner, Swiss Centre for Applied Ecotoxicology, Eawag/EPFL /

Department of Anatomy, Physiology and Cell Biology. Environmental quality standards (EQS) are used under the EU Water Framework Directive to determine the chemical status of a water body. So far, compliance with EQS is only checked on the single substance level. However, vast evidence from mixture toxicity studies indicates that this procedure might underestimate the toxicity of the chemicals present in a water body, as the effect of mixtures was shown to be higher than the highest single substance effect in the majority of cases. In order to take this into account mixture toxicity assessment can be incorporated into EQS compliance checking. For substances with the same mode of action Toxic Units (TUs = Env. Conc./EQS) can be added. However, similarity of action is usually difficult to assess and the summation of substance-specific TUs is likely to overestimate the mixture toxicity, because individual EQS are not all based on the same taxonomic group. Backhaus and Faust (2012) have proposed a tiered approach for mixture toxicity under the EU REACH legislation. Thereby, for each trophic level the acute toxicity of all substances in a mixture is predicted ( $\Sigma$ TU). From the resulting risk quotients for algae, daphnids and fish the highest  $RQ_{\Sigma TU}$  is then chosen and multiplied with an assessment factor (AF) of 1000. While this is a sound approach under REACH, where data availability is often limited to the "base set", it can hardly be applied on EQS which are usually based on more diverse input data (i.e. chronic data, SSD method, mesocosm data). Hence, a more pragmatic approach is needed. Accordingly, we propose the  $RQ_{EQS-taxa}$  summation, which takes into account that EQS derivation is driven by the most sensitive taxonomic group(s). This approach, was applied to a water quality assessment based on real monitoring data of ambient surface water samples. Therefore EQS dossiers were compiled for as many measured substances as possible and assigned to the different taxonomic groups. To test if the  $RQ_{EQS-taxa}$ -summation underestimates the mixture toxicity, the results were compared to EQS derived with the same data set applying the  $RQ_{\Sigma TU}$  approach (Backhaus and Faust 2012). The proposed  $RQ_{EQS-taxa}$ -summation was shown to be a pragmatic and easy-to-apply method for considering mixture toxicity, when EQS are based on pelagic species, irrespective of whether the EQS was derived based on the AF, the SSD or the mesocosm approach. It was shown to give sound assessments of the water quality.

#### **RP124 Advancing Risk and Hazard Assessment of Fragrance Materials**

I. Davies, Research Institute for Fragrance Materials, Inc.; A. Lapczynski, Research Institute for Fragrance Materials, Inc.; Research Institute for Fragrance Materials, Inc. / Environmental Specialist; D. Salvito, Research Institute for Fragrance Materials, Inc. / Department of Environmental Science; C. Sachse Vasquez, Research Institute for Fragrance Materials, Inc. The Research Institute for Fragrance Materials (RIFM) provides environmental risk and hazard assessment of fragrance ingredients used in consumer products. This has evolved into an extensive research and testing program supporting a better understanding of the environmental behavior of fragrance materials in the environment, the development and implementation of the IFRA Environmental Standards, and multiple regulatory programs on behalf of the industry (e.g., REACH, DSL). Presented here is a summary of the current state of the program, efforts underway to complete all hazard assessment within a five year horizon, and the integration of these activities into the RIFM database (a relational database of material safety information available to industry users).

#### **RP125 Baseline Ecological Risk Assessment of Metals and Radionuclides Utilized for Remedial Planning at an Abandoned Uranium Mine**

B. Milne, M. Sanborn, AECOM Canada Ltd.; D. Bright, Hemerra; T. Yankovich, A. Klyashutorin, SRC. The Gunnar Mine was a uranium mine that operated in Northern Saskatchewan, Canada, between 1953 and 1964 and at the time of closure was abandoned with little to no attempts at decommissioning or reclamation. The Site is located on the shore of Lake Athabasca and consisted of a town-site, mine buildings, open pit, underground workings, headframe, ancillary buildings, unconfined tailings, and waste rock. The mine sat abandoned for decades and as a result, the residual tailings and waste rock have lead to concerns regarding metals, uranium and U-series radionuclides in the surrounding environment. The Gunnar Site remediation is being managed by the Saskatchewan Research Council (SRC) on behalf of the Governments of Saskatchewan and Canada as part of SRC's Cleanup of Abandoned Northern Sites (CLEANS) program. An environmental risk assessment was conducted to develop risk-based remedial solutions based on both current site conditions and predicted conditions for a variety of remedial options. In order to evaluate species that were

ecologically significant or had economic and/or social value, ecological receptors were chosen based on consultation with northern residents incorporating traditional knowledge and land use activities. Exposure assumptions included dietary composition and food intake rate, soil/sediment ingestion rate, water ingestion rate and time the receptor was expected on site. Contaminant concentrations were directly measured in a variety of matrices (i.e. soil, water, vegetation, small mammal and fish tissue). In the absence of site-specific tissue data, concentrations were calculated using measured data and contaminant specific transfer factors. Radionuclide exposure was calculated taking into consideration the absorbed dose (intake), the equivalence dose (the linear energy transfer (LET) of the ionizing radiation), the internal dose (uptake) and the relative biological effectiveness (RBE) of the radiation. Two radiation benchmarks were chosen from pertinent literature for both aquatic and terrestrial receptors in order to show the uncertainty in the literature. Biota exposure to uranium and select metals exceeded benchmark values for aquatic receptors, waterfowl and mammals reliant on aquatic vegetation, and benthos. Radionuclide exposure exceeded benchmarks for all aquatic receptors. Remedial options are being developed by AECOM and SRC to effectively mitigate risks to terrestrial and aquatic ecological receptors.

#### **RP126 Bringing Submerged Aquatic Vegetation Back to Starved Rock Pool: Application of Risk and Uncertainty Analysis and Enhanced Adaptive Management**

J.T. Vogel II, K. Baker, U. S. Army Corps of Engineers / Engineer Research and Development Center; B.C. Suedel, U.S. Army Engineer Research and Development Center / CEERD-EP-R, US Army Engineer Research and Development Center / Environmental Laboratory, Waterways Experiment Station EP-R; M. Plumley, C. Haring, U. S. Army Corps of Engineers; I. Linkov, US Army Corps of Engineers / Engineer Research and Development Center, US Army Engineer Research and Development Center. Risks and uncertainties inherent to ecosystem restoration efforts pose significant challenges to selecting optimal alternatives and avoiding unintended adverse affects. Efforts to overcome these challenges necessitate a planning framework that allows practitioners to account for uncertainties in initial alternative selection and to quickly adapt to new information by updating project measures as knowledge is gained. In light of this, USACE regulatory guidance calls for ecosystem restoration planners and practitioners to incorporate risk and uncertainty (R&U) characterization and adaptive management planning into restoration efforts. To address this, our team has developed and applied an Enhanced Adaptive Management (EAM) framework that integrates risk and uncertainty analysis, adaptive management, and decision analytics. Given that the US Army Corps of Engineers (USACE) is projected to spend \$512 million on ecosystem restoration in Fiscal Year 2013, restoration efforts are clearly a national priority, and the techniques addressing risk and uncertainty and project implementation covered in this poster presentation can lead to improved decision making and a more efficient use of limited resources. We review the risk and uncertainty analysis methodology, adaptive management plan development, including targeted monitoring and analysis, and finally the decision analytic approach highlighting how these tools and techniques can be used to better inform decision-making. The poster continues with a case study in which we apply the EAM to the USACE Rock Island District's project aimed at restoring submerged aquatic vegetation (SAV) in Starved Rock Pool, a 16 mile stretch of the Illinois River. We examine three types of uncertainty, parameter, scenario, and model uncertainty, to give a broader characterization of the risks involved and apply a decision model that incorporates hydraulic, wind fetch and wave, and submerged aquatic vegetation models to determine the effects of each alternative and select the optimal alternative initially and as new information is discovered. The long term goal of this project is to develop an uncertainty analysis that informs an adaptive management plan for SAV restoration that prioritizes monitoring efforts and provides an iterative management and restoration process.

#### **RP127 Ecological Risk Assessment for Mercury, Arsenic, Antimony, and Other Metals at an Abandoned Mercury Mining and Processing Site in Interior Alaska**

C. Mach, Ecology and Environment, INC. / Department of Environmental Sciences, Ecology and Environment, INC.; W. Richards, M. Longtine, E & E; M. McCrum, M. Varner, Bureau of Land Management. The Red Devil Mine (RDM) site is an abandoned Hg mining and processing facility located adjacent to the Kuskokwim River in Interior Alaska, approximately 250 miles west of Anchorage, Alaska. It is located on public lands managed by the United States Department of the Interior

(DOI) Bureau of Land Management (BLM). The RDM was Alaska's largest Hg mine, producing 1.2 million kg of Hg between 1933 and 1971. Cinnabar (HgS) and stibnite (Sb<sub>2</sub>S<sub>3</sub>) were the principal minerals associated with the ore zone, with minor amounts of realgar (AsS), orpiment (As<sub>2</sub>S<sub>3</sub>), and pyrite (FeS<sub>2</sub>) also locally present. Contaminated soil, tailings, overburden, and other wastes from the RDM have been exposed at the surface for decades. Hg and other metals in these wastes have been subject to transport by water and wind to a small on-site creek, the Kuskokwim River, groundwater beneath the site, and surrounding terrestrial areas. A baseline ecological risk assessment was conducted for the RDM site as part of the Remedial Investigation/Feasibility Study (RI/FS). Soil, sediment, surface water, groundwater, and plant tissues were collected and analyzed for Hg and other site-related contaminants for the RI/FS. Hg and As speciation were determined on a subset of samples of most media. The soluble fraction of Hg and other metals in contaminated soils and mine wastes also was investigated. Fish and benthic macroinvertebrates were collected and analyzed for Hg and other site-related contaminants for a concurrent investigation conducted by the BLM. Potential risks to ecological receptors in terrestrial and aquatic habitats at and near the site were evaluated using the available data. This poster will describe the site habitats and receptors, ecological conceptual site model, study design, analytical results, risk results and conclusions, and uncertainties.

**RP128 Ecotoxicity and Accumulation of Rare Earth Elements** M. Whitfield Aslund, Intrinsik Environmental Sciences Inc., University of Toronto; R.N Hull, Intrinsik Environmental Sciences Inc.; L.J. Marshall, Intrinsik Environmental Sciences / Environmental Scientist; C. Moore, K. Rankin, Intrinsik Environmental Sciences Inc.. Rare earth elements (REE) are the 15 elements of the lanthanide family plus scandium and yttrium, which have similar properties to the lanthanides. The REE are receiving significant attention because they are important components of modern technological instruments, such as smart phones, electrical vehicles and wind turbines. However, few environmental quality standards exist for these elements, and few aquatic or terrestrial ecotoxicity studies have been conducted. Some acute aquatic toxicity data suggest high toxicity of these elements. However, very little chronic or terrestrial toxicity data exist. Several elements are of relatively low bioaccumulation potential. The available toxicity and accumulation data for the REE will be presented and discussed.

**RP129 Environmental Risk Assessment of Sulfamethoxazole in Japan based on the European Medicines Agency Guideline** R. Nabeoka, M. Seki, T. Kayashima, Chemicals Evaluation and Research Institute, Japan. Antibiotics and antimicrobial are used widely to treat bacterial infections. Sulfamethoxazole (SMX), one of the antimicrobials, has been found in wastewater treatment plant effluents and surface water in Japan. Nevertheless, no environmental risk assessment of SMX has been performed. The purpose of this study was to assess an environmental risk of SMX in Japan. The environmental risk assessment of SMX was performed according to "Guideline on the Environmental Risk Assessment of Medicinal Products for Human Use" by European Medicines Agency. In Phase I as an initial assessment of environmental exposure, the initial predicted environmental concentration (PEC) of SMX in surface water was calculated as 8.0 µg/L which exceeded the action limit of 0.01 µg/L. In the next step, initial environmental fate and effect analysis were performed in Phase II Tier A. Outcome of Phase II Tier A indicated that SMX had a low potential to affect on sediment and terrestrial organisms. In addition, it was indicated that SMX had a low concern to affect on organisms in groundwater and microorganisms in sewage treatment plant. Furthermore, it was shown that SMX had a low potential for bioaccumulation. However, PEC for surface water exceeded the predicted no-effect concentrations (PNEC). Accordingly, we refined the PEC value of SMX in surface water by using the following data in Japan: production of SMX, its removal rate at a sewage treatment plant, its photodegradation rate, dilution factor of treated wastewater, and the amount of wastewater per day per capita. As a result, the refined PEC for surface water was 0.072 µg/L which was below the PNEC of 0.59 µg/L, indicating low concern of SMX for surface water. Overall, we conclude that the level of environmental risk of SMX in Japan was acceptable.

**RP130 Examining the fate and effects of vanadium in light of the USEPA Framework for Metals Risk Assessment** C. Menzie, Exponent, Inc., Exponent / EcoSciences; L. Ziccardi, Exponent / EcoSciences; Y. Lowney, Exponent. When conducting human health and ecological risk

assessments for vanadium, as with other metals, it is important to consider the environmental chemistry, chemical forms, and exposure routes. Prior research has shown that the toxicity of vanadium depends on its physico-chemical state, particularly on its valence state and solubility, and toxicity generally decreases as vanadium undergoes chemical reduction. While there is information on the toxicity of different forms of the metal (i.e., vanadium species and compounds), there is a poor understanding of vanadium forms that occur in environmental media and how this relates to available toxicity data. This information gap for exposure presents a challenge for selecting the appropriate toxicity values for risk assessments. The chemical forms that serve as the basis for established regulatory toxicity values may not be relevant to the forms found in the environment. The U.S. Environmental Protection Agency's regulatory guidance on the risk assessment of metals advises risk assessors to consider such factors as environmental chemistry, naturally occurring background levels, and beneficial effects. Our review of the existing literature suggests that risk assessors have room to improve the way these factors are considered when evaluating the risks of vanadium.

**RP131 Leaching Analysis and Environmental Risk Assessment of 2-Methyl-1,2-benzisothiazolin-3-one as an In-can preservative for Architectural Coatings** J.P. Carbone, Dow Chemical Company / Toxicology, Environmental Research and Consulting, The Dow Chemical Company / Toxicology, Environmental Research and Consulting; D. Laganella, The Dow Chemical Company / Dow Microbial Control; J. Ryder, The Dow Chemical Company; G. Hazelton, The Dow Chemical Company / Toxicology, Environmental Research and Consulting. The environmental risk of 2-Methyl-1,2-benzisothiazolin-3-one [mBIT] was assessed based on the use of the biocide as an in-can preservatives for architectural coatings. The risk analysis entailed examining direct deposition of leached biocide into a modeled surface water body simulating an urban scenario where runoff occurred via impervious surfaces. A second scenario entailed the leaching of the biocide and transport to an adjacent water body across pervious, soil surfaces. The later scenario would simulate a suburban scenario. Leaching of the biocide was measured via the OECD [Short Duration] method where coated matrices of measured surface area were exposed intermittently to a defined volume of water. A leach rate was determined and reflected mass leached/unit area/mm water. Leach rates were interfaced with the USEPA weather data set for Jackson, MS. The modeled environment was the Mississippi corn scenario modified to reflect a non-cropped landscape such that foliar interception of biocide and water were negligible. Mass load/unit area/rainfall [mm] values were calculated based on the current USEPA risk assessment paradigm used in conjunction with the Jackson, MS weather set. The resulting mass load data was input into the USEPA fate and transport model PRZM v 3.12.2.0 which was interfaced with EXAMS v 2.98.4.6 a surface water model employing the PERL interface. The USEPA SCI-GROW model was used to estimate potential environmental concentrations in soil pore water. Based on calculated risk quotient values mBIT would not exert adverse effects on soil nitrification and mineralization process. Similarly, the probability of mBIT adversely affecting terrestrial plant species is considered negligible. The potential of adverse effects to terrestrial macroinvertebrates such as the earthworm has also been shown to be negligible. With regard to aquatic organisms, the resulting calculated RQ values were consistently below the presumptive risk levels of concern. Evaluation of the potential for groundwater contamination using SCI-GROW indicated that the likelihood of mBIT contamination of aquifers was negligible.

**RP132 Partitioning of Organic Compounds in Complex Organic Mixtures Lacking Full Compositional Information** D.T. Kuo, University of Delaware / Department of Civil and Environmental Engineering; U. Kipka, Anchor QEA, LLC, Anchor QEA. The fate and toxicological assessment of organic contaminants in natural environments often depends on understanding their partitioning behavior in various environmental compartments. Model compartments such as complex petrogenic liquid or solid mixtures at impacted sites are of particular concern as they affect bio-availability estimates. For systems where exact chemical composition of the mixture is unknown, a novel approach will be needed. This work evaluates several plausible alternatives for models that approximate organic mixtures with generic organic surrogate molecular structures and combinations of surrogate structures. A similar approach has previously been attempted for humic material in rivers, and soil organic carbon. We evaluate the approach using experimental data on crude oil and other non-aqueous phase liquid mixtures and relate model compartment chemistry with laboratory



characterizations of petrogenic mixtures. In particular, we believe this work is relevant for the assessment and remediation of weathered or long deposited complex organic liquid mixtures where full compositional characterization may be difficult to obtain.

#### RP133 Regional Impacts of the Deepwater Horizon Oil Spill on

**Gulf of Mexico Ecosystem** M. Li, University of Michigan / School of Natural Resources and Environment, Harvard School of Public Health / Environmental Health; M. Thurman, Harvard School of Public Health; E. Sunderland, Harvard University of Public Health; E. Chesney, Louisiana Universities Marine Consortium; D. Senn, San Francisco Estuarine Institute; J.P. Shine, Harvard School of Public Health / Department of Environmental Health. The Deepwater Horizon blowout, one of the largest offshore oil spills in human history as well as the first one occurring at significant depth, released a large amount of pollutants from a deep ocean oil reservoir into the Gulf of Mexico marine ecosystem in the summer of 2010. This study aims to identify and interpret changes of chemical bioaccumulation (PAHs and Hg) in coastal and oceanic fishes in Gulf of Mexico before and after Deepwater Horizon oil spill. A large number of fish samples from south of Terrebonne Bay, LA were collected both in 2006 and several months after the oil release in 2010. Samples included various fish species, representing a range of habitats, life histories, prey preferences, and literature-predicted trophic positions based on diet. Contrary to our hypothesis that the oil spill would result in elevated concentrations of PAHs in fish, the observed total concentrations of PAHs were lower after the oil spill in 2010 relative to 2006, with a different fingerprint relative to pyrogenic or petrogenic sourcing. The comparison of total Hg in fish tissue before and after oil spill showed that Hg levels increased significantly for some coastal fish species such as Gulf Menhaden and Redfish but decreased for some migratory species like Spanish Mackerel. These results imply that changes in the food web of Gulf of Mexico may have occurred due to the oil spill, which affected bioaccumulation of contaminants in higher trophic level species. Stable Carbon and Nitrogen isotopic data, as well as a food web bioaccumulation model contribute further lines of evidence for the observed changes.

#### RP134 Use of QSAR Validation Principles to Enhance Predictive Approaches in the US EPA ECOSAR Model

C. Russom, US EPA / Office of Research and Development, NHEERL, Mid-Continent Ecology Division; K. Mayo-Bean, K. Moran, US EPA / Office of Pollution Prevention and Toxics; K. Eisenreich, University of Maryland Center for Environmental Science: Chesapeake Biological Laboratory / UMCES-CBL, US EPA / Office of Pollution Prevention and Toxics. The US EPA Office of Pollution Prevention and Toxics (OPPT) is responsible for implementing the Toxic Substances Control Act (TSCA). TSCA is the US law that regulates industrial chemicals in the US and OPPT evaluates both new chemicals entering commerce, as well as those chemicals that have been in existence for some time. In evaluating new and existing chemicals over the past 35+ years, OPPT has developed a strong knowledge base in structure-activity relationships (SARs). The Ecological Structure Activity Relationships (ECOSAR) Class Program is a computerized predictive system that estimates aquatic toxicity. The program estimates a chemical's acute (short-term) toxicity and chronic (long-term or delayed) toxicity to aquatic organisms based on their structural similarity to chemicals for which aquatic studies are available. Model development is continuous and dependent on availability of new data and information submitted to the US EPA or generated within the scientific community. Recent work was completed in ECOSAR version 1.1 to better represent toxicity data of pesticidal compounds within the model and enhance the chemical space of the tool. Following the OECD QSAR validation principles, the US EPA's Office of Research and Development conducted an independent validation of the ECOSAR version 1.1 QSAR models using a set of chemicals having an acute mode of action of acetylcholinesterase inhibition. The resulting case study titled 'Use of ECOSAR QSAR Models to Estimate the Acute Toxicity of Organophosphate and Carbamate Pesticide Classes to Fish Species' found that the carbamate esters performed reasonably well, but highlighted the need for improvement of chemical categories and models for the phosphate esters. EPA reviewed additional data, chemical diversity, and use classes (e.g., pesticidal activities versus phosphate esters used in industrial applications like flame retardants) within the training sets to identify ways to improve the QSAR relationships. The US EPA will present these results along with updated QSAR regression analysis for the phosphate esters. *Disclaimer: This abstract represents the views of the authors and not necessarily the official position of the US EPA.*

#### RP135 Using Chronic Aquatic Toxicity Data in the Risk Assessment of Phthalate Ester Plasticizers

C.A. Staples, Assessment Technologies, Inc.; R. Guinn, Eastman Chemical Company / Corporate Health, Safety and Environment Dept.; K. Kramarz, BASF Corporation; M. Lampi, ExxonMobil Biomedical Sciences, Inc.; N. Scholz, Evonik Oxeno GmbH. Phthalate esters are a class of chemicals varying greatly in terms of uses, properties and toxicity. C1 to C4 phthalate esters are used in non-vinyl commercial products and pharmaceuticals. C8 to C10 phthalate esters are additives imparting flexibility to vinyl resins. The purpose of the present study is to assess chronic effects of phthalate esters on aquatic organisms. Studies show that populations of fish and invertebrates may be adversely affected by exposure to C1 to C4 phthalate esters, but are not adversely affected by exposure to C8 or higher phthalate esters. Secondary endpoints, including molecular, biochemical, and/or histological responses to chemical exposure, do not appear to predict effects related to primary endpoints of survival, growth and development, or reproductive fitness. A previously published risk assessment for C1 to C4 phthalate esters demonstrated low risks in North American and Western European surface waters. Risk assessments conducted by authorities in Europe with DEHP, DINP, and DIDP have concluded no risks to aquatic organisms due to aqueous solubility constraints, low expected surface water concentrations, and metabolic biotransformation capabilities of aquatic organisms. Important chronic aquatic toxicity studies have been conducted since those assessments and are presented here. The data from the present studies, support the earlier risk assessment conclusions. The data also provide further support for a narcosis-related aqueous solubility cutoff at approximately C6 and higher phthalate esters. Finally, for the C1 to C4 phthalate esters, the present study shows that secondary endpoints (e.g., molecular, biochemical, and/or histopathology) to date, provide limited benefit in practical ecological risk assessment of phthalate esters to aquatic species.

#### RP137 Research on the Migration of Young Green Turtles (Chelonia Mydas) in the ArcelorMittal Tubarao Industrial Effluent

J.O. Santos, ArcelorMittal Tubarão / Environment; F. Passamani, ArcelorMittal Tubarão / Environment. The green turtle – *Chelonia Mydas* – is globally distributed in tropical and subtropical seas. In Brazil, their reproduction areas are located in the oceanic islands of Fernando de Noronha, Atol das Rocas and Trindade Island. Young and adult specimens are found in feeding areas along almost the entire coast of Brazil. This species, as well as the other four species existing in the Brazilian coast, is part of the list of endangered fauna species in Brazil, also considered endangered by the World Conservation Union. The research institution – Projeto TAMAR-IBAMA – has acted especially on the main reproduction areas in Brazil since 1980, and in 1990 it started working in the feeding areas. Biometric and marking works are carried out in the reproduction and the feeding areas by the institution, aiming to better understand the biological and migration standards of this species, which serve as basis for preservation activities. In 2000 a technical cooperation agreement was signed between the research institution and ArcelorMittal in order to carry out studies on the biology of the species in the area of influence of the company. Field works have been performed since August 11<sup>th</sup>, 2000 with the main objective of carrying out biometric studies by capturing and marking them to record growth, migration patterns, haematological profile and health conditions of the green turtles that use the marine water of ArcelorMittal Tubarão effluent area. ArcelorMittal Tubarão is located 14km north of Vitoria (State of Espírito Santo, Brazil). Treated domestic and industrial effluents from the company are thrown in one single marine area (final effluent). Water and sediments receive physical-chemical and toxicological monitoring every six months and a biological monitoring is also carried out examining the bioaccumulation of heavy metals in bivalve molluscs. The area studied is located at the end of the effluent channel and has an average temperature 6° above sea water temperature, which, together with the availability of organic matter, favours an environment rich in algae. This study presents the monitoring results from 2009-2010 including field methods and data collection results from the effluent area.

#### RP138 A reproduction toxicity test of CuO-Nanoparticle and copper(II) chloride dihydrate to Paronychiurus kimi (collembola)

S. Yu, Korea Univ.; Y. Lee, Korea University / Division of Environmental Science and Ecological Engineering, College of Life and Environmental Sciences; Y. Kim, Korea University / Division of Environmental Science and Ecological Engineering, College of Life and Environmental Sciences; H. Mo, Korea University / Division of Environmental Science and Ecological Engineering; K. Cho, Korea University / Division of Environmental Science and

Ecological Engineering, College of Life and Environmental Sciences. There have been many studies of nanoparticle (NP) affecting on water system, but few on terrestrial system. The objective of this study was to determine the chronic toxic effect of CuO-NP on *Paronychiurus kimi* in the OECD artificial soil. The test chemicals, 30 min sonicated CuO-NP, copper(II) chloride dihydrate and CuO-NP with two surfactants, Polyallyl amin hydrochloride (PAH) and poly acrylic acid (PAA) which were used for dispersion of CuO-NP, were spiked to of OECD soil test media for 28-days. The tested concentrations of copper was 1000, 750, 500, 250, 125 mg/kg, PAH and PAA were 5 mg/kg and 50 mg/kg, respectively. Survived adults and hatched juveniles were counted after 28-day exposures to investigate reproduction level. The survived adults were used as samples for measuring the internal body concentration and investigating effect on reactive oxygen species. Result of this study provide basic information of effect on the Collembola in NP treated soil and it will help to assess the toxic effect on terrestrial organisms.

**RP139 An evaluation of the effects of multiwalled carbon nanotubes on bioavailability and phytoremediation of polycyclic aromatic hydrocarbons (PAHs)** B. Shrestha, Texas Tech University / Department of Environmental Toxicology, Texas Tech University; T.A. Anderson, Texas Tech University / Environmental Toxicology; P. Payton, USDA-ARS; Q. Cai, Texas Tech University / TIEHH / Environmental Toxicology; J. Canas, Texas Tech University / Department of Environmental Toxicol. Rhizodegradation is considered to be an effective phytoremediation method for the remediation of contaminants like PAHs from the soil. Rhizodegradation is dependent on contaminant bioavailability to plant rhizosphere microorganisms. Bioavailability of organic contaminants like PAHs can be decreased due to sorption and sequestration to the soil. Carbon nanotubes (CNTs) are considered to be a good adsorbent for organic contaminants like PAHs. Although CNTs are good adsorbent for PAHs, studies have found that desorption of PAHs from CNTs is also high. Hence, CNT presence can affect PAHs bioavailability in soil by adsorption and desorption. This study aimed to compare the effects of multiwalled carbon nanotubes (MWNT) on PAHs bioavailability for rhizodegradation and overall phytoremediation in two different soil types (sandy loam (SL) and clay loam (CL) with added potting mix to increase organic matter content). Soil was first spiked with PAHs (pyrene and phenanthrene) at 100 mg/kg and then with MWNT at 0, 25, 50, and 100 mg/kg. Alfalfa plants were grown in pots containing PAHs and MWNT for 49 d. Soil was extracted using accelerated solvent extraction and analyzed using GC-MS at the beginning and end of the experiment. Germination of alfalfa was not significantly affected in both soil types; however average germination was comparatively higher in CL controls. Alfalfa shoot and root biomass were not affected in any treatment in SL soil. Also, there was no significant difference in PAHs residues among all MWNT treatment groups, suggesting no effects of MWNT on PAHs bioavailability in SL soil. In this study, pyrene and phenanthrene degraded more than 97% and 99% in all treatments in SL soil which shows that PAHs were highly bioavailable in the SL soil regardless of MWNT treatment probably due to low organic matter content (1%) and clay content (15%). Hence, the experiment was repeated with CL soil to see if a similar trend will be observed in soil containing high organic matter and clay content. Microbial community composition, respiration and dehydrogenase activity was also monitored to determine effects of MWNT on rhizosphere microbial community. This study expects to provide important data on the effects of MWNT on PAH bioavailability in different soil types and its potential application in phytoremediation of contaminated sites.

**RP140 Behavior as an indicator of impacts from carbon nanomaterial on *Daphnia Magna*** A.J. Nikolaus, University of Wisconsin-Milwaukee / Great Lakes Water Institute; R. Klaper, University of Wisconsin-Milwaukee / School of Freshwater Sciences, University of Wisconsin-Milwaukee / Great Lakes Water Institute. As the use of carbon nanomaterials across multiple industries grows so too does the need to understand the possible impacts that they may have on freshwater organisms. More specifically, understanding the impacts on behavior that may occur in response to nanomaterial exposure is important as deviations in standard behavior can lead to changes in predation and in feeding rate. Most studies have shown low acute toxicity but few have been done on low-level impacts for other responses such as the behavior of organisms. Our previous studies have found that exposure to both functionalized and unfunctionalized carbon fullerenes caused changes in hopping behavior and heart rate in *Daphnia*, a freshwater crustacean. In

this study we evaluated a higher throughput method of assessing behavioral changes in response to exposure to a wide variety of nanomaterials of varying surface chemistries. Concurrent studies are also evaluating the mortality and possible reproductive alterations caused by nanomaterial exposure. Of the materials tested single walled carbon nanotubes (SWNT) functionalized with polyethylene glycol (PEG) showed the most significant change in velocity at a concentration of 50 parts per million. When exposed during light SWNT-PEG and the carbon fullerene C60-D4 showed significant changes in the *daphnia's* total time spent mobile. In some cases our data shows a direct link between behavior and traditional apical endpoints indicating a potential for the use of behavior as a more sensitive and rapid indicator of effects.

**RP141 Characterizing the bioavailability of fluoranthene adsorbed to carbon nanotubes in the presence of natural organic matter** E.N. Linard, Clemson University / Institute of Environmental Toxicology (ENTOX); S.J. Klaine, Clemson University / Institute of Environmental Toxicology (CU-ENTOX), Clemson Institute of Environmental Toxicology / Institute of Environmental Toxicology (ENTOX), Clemson University / Institute of Environmental Toxicology (ENTOX); P. van den Hurk, Clemson University / Biological Sciences. The introduction of carbon nanomaterials into the environment has increased exponentially in the last decade, causing environmental as well as health concerns. Of particular concern is the interaction that such nanomaterials have with the biota in the aquatic ecosystem and the direct and indirect toxic effects that may result. Previous research has documented positive influence of natural organic matter (NOM) on the stability of carbon nanotube (CNTs) suspensions in surface waters. Further, other research has quantified the ability of these nanomaterials to adsorb aquatic contaminants such as polynuclear aromatic hydrocarbons (PAHs). It is important to note that both CNTs and PAHs can co-occur in wastewater treatment effluents. However, no research has characterized the bioavailability of these adsorbed PAHs. The goal of this research was to characterize the bioavailability of fluoranthene that has been adsorbed to CNTs. Adsorption and desorption isotherms for fluoranthene and CNTs in the presence of varying NOM concentrations were developed. *Pimephales promelas* were exposed for 48 hrs to different treatments including fluoranthene alone, fluoranthene in the presence of different concentrations of NOM, and fluoranthene adsorbed to CNTs in the presence of NOM. Bioavailable fluoranthene was quantified in each exposure through bile analysis using a fluorescence microplate reader. Our preliminary results indicate that fluoranthene is readily bioavailable in waters without NOM and that this bioavailability decreases with increasing NOM. Results of this work will be used to develop a model to predict this complex interaction.

**RP142 Comparison of multi-walled carbon nanotube chronic toxicity and bioaccumulation in two crustaceans: freshwater and estuarine** E.R. McReynolds, University of Georgia / Environmental Health Science; M.C. Black, University of Georgia / Dept. of Environmental Health Science. Multi-walled carbon nanotubes (MWCNTs) have infinite uses in the biotechnology field. With cheaper production, they are produced on a large scale with the potential to contact the aquatic environment through product use and degradation or accidental or known releases. Although products containing MWCNTs are currently available on the market, very little toxicity data are available for both saltwater and freshwater organisms. The objectives of this study were to measure chronic toxicity and bioaccumulation of MWCNTs in two crustaceans adapted to different aquatic environments: freshwater (*Ceriodaphnia dubia*) and estuarine (*Americamysis bahia*). In separate experiments, *C. dubia* and *A. bahia* were exposed to a range of MWCNT concentrations over a 7 d period (1.25-5 mg/L, 0.1-10 mg/L, respectively). Chronic toxicity endpoints for *C. dubia* exposures were mortality and reproductive output. Endpoints for *A. bahia* exposures were survival, maturation to reproductive adult and offspring production. Nanotubes were labeled with to facilitate measuring their accumulation. No significant mortality of either organism was noted at any concentration. Reproductive output was significantly decreased (LOEC) by *C. dubia* at 2.5 mg/L MWCNTs. There were no significant reductions in reproductive output in *A. bahia* compared with controls, but a 59.5% decrease in the number of mature individuals was observed at 5 mg/L. Both organisms accumulated MWCNTs following exposure to 5 mg/L. Adult *C. dubia* accumulated 14.69±5.9 ng MWCNTs/organism while the larger organism, *A. bahia*, accumulated 25.08±6.3 ng/organism. We hypothesize a similar mode of action for reproductive toxicity of MWCNTs in estuarine and freshwater



aquatic invertebrates. The physical adherence of the MWCNTs to both *C. dubia* and *A. bahia* may have caused disruption of feeding appendages and inhibition or disruption of the molting process, resulting in fewer offspring (*C. dubia*) and reduced maturation (*A. bahia*). This is the first research to compare toxicity of MWCNTs between two distinct aquatic invertebrates.

**RP143 Deriving guideline of gold nanoparticles to protect aquatic life** Y. An, Konkuk University / Department of Environmental Science, Konkuk University / School of Earth and Environmental Sciences, Seoul National University / School of Earth and Environmental Sciences; S. Nam, Y. Shin, S. Yoon, W. Lee, S. Kim, J. Kwak, Konkuk University. This study conducted a battery of bioassay for gold nanoparticles to derive guideline value to protect aquatic ecosystems. Test species were two bacteria (*Escherichia coli* and *Bacillus subtilis*), one green algae (*Pseudokirchneriella subcapitata*), three cladocerans (*Daphnia magna*, *Moina macrocopa* and *Simocephalus mixtus*), and two fish (*Oryzias latipes* and *Danio rerio*). Predicted no effect concentration (PNEC) was estimated to be 0.04 mg/L for gold nanoparticles based on species sensitivity distribution (SSD). To the best of our knowledge, this is the first result to suggest water quality guideline value of gold nanoparticles to protect aquatic life. *This work was supported by the National Research Foundation Grant funded by the Korean Government (NRF 2011-0015985).*

**RP144 Determination of carbon nanotube bioaccumulation in earthworms measured by a microwave-based method** S. Li, Texas Tech University/The Institute of Environmental and Human Health / Environmental Toxicology, TIEHH; F. Irin, F. Atore, M. Green, J. Canas, TTU. Carbon nanotubes (CNTs) are one of the mostly widely developed nanomaterials with various applications. However, current studies of CNT fate and toxicity are impeded by the lack of a reliable quantification method. This study aimed to develop a novel method to determine multiple-walled carbon nanotube (MWNT) concentrations in earthworms. This study was an expansion of a previously developed microwave-induced heating method for MWNT detection in plants. In this method, MWNT heat generation exposed to microwaves was correlated with MWNT mass in the sample. With the lowest reported detection limit for MWNTs (0.1 µg per earthworm sample), the microwave-induced heating method has the potential to quantify MWNTs at environmentally relevant concentrations with some further modification. With the aid of this novel method, biouptake and elimination behavior of MWNTs in earthworms (*Eisenia fetida*) was evaluated. A bioaccumulation factor of  $0.015 \pm 0.004$  indicated that MWNTs did not bioaccumulate in earthworms.

**RP145 Ecotoxicological Effects of Nanometer Zinc Oxide on Soil Microorganisms** X. Lu, Peking University / Lab for Earth Surface Processes, College of Urban and Environmental Sciences; Z. Chen, Z. Shen, Peking University / College of Urban and Environmental Sciences; S. Zhang, Peking University; Z. Hou, J. Yang, Peking University / College of Urban and Environmental Sciences. With the rapid development of nanotechnology, manufactured nanomaterials are increasingly used and may contaminate the soil during the process of their use. Nanometer Zinc Oxide (NZO) is one of the most used nanomaterials due to its fine properties. The toxicity of NZO on pure cultures has been studied by some researchers, however, little is known about their effects in complex matrix. This study aimed at investigating the ecotoxicological effects of NZO on soil microorganisms. Fresh soil from a hillside was collected for the experiment. NZO with mean particle diameter of 20 nm and purity of 99% was purchased from a company. Using self-designed apparatus, the NZO was mixed with the soil at concentration of 1 mg NZO per gram of fresh soil and then split into 18 Erlenmeyer flasks. Fresh soil without NZO was used as control and also split into 18 Erlenmeyer flasks. All the flasks were covered with gauze and incubated in a cool and dark closet. Periodically, distilled water was added to each of the flasks to maintain the soil water content at about 5%. Every four weeks, three flasks of the NZO treated soil and three flasks of the control soil were taken to analyze different ecotoxicological parameters including microbial biomass, biochemical processes (respiration and ammonification) and activities of enzymes (dehydrogenase and fluorescein diacetate esterase). After three months of experiment, the results showed that there was no significant differences in values of the tested parameters between the NZO tested soil and the control soil. We hypothesized that the bioavailability of NAO was greatly reduced when it was mixed with the soil. To test this hypothesis, NAO was added to the soil turbid liquid (soil water ratio of 1:10) at concentration of 1mg/L and incubated for two weeks. The results showed

that the activity of fluorescein diacetate esterase was inhibited but no change was observed for other parameters. Then we used pure culture isolated from the soil to test the toxicity of NZO at different concentrations. It turned out that the presence of NZO reduced the pure culture's biomass and enzyme activities and these effects were positively correlated with the NZO concentrations. No inhibition on respiration was observed under the test condition. In conclusion, bioavailability limits the ecotoxicological effects of NZO on soil microorganisms.

**RP146 Effects of aqueous metal-oxide nanoparticles on RBL-2H3 cell cytokine secretion** V.A Ortega, University of Alberta / Department of Biological Science; J.D. Ede, J.L. Stafford, G.G. Goss, University of Alberta / Department of Biological Sciences. Nanotechnology is a multidisciplinary field that involves the synthesis of molecules in the nanoscale (< 100nm). The small size of nanoparticles (NP) produces unique physico-chemical properties that are different from their larger bulk forms and have thus generated interest for their potential to enhance industrial processes. However, there is growing concern over the potential and unknown toxicity of these particles on biological systems. The effects of NPs on cellular toxicity have been studied to a large extent with evidence showing impacts on cell stress and apoptotic pathways. However, the effects on the immune system and immune cells is still largely unstudied and remains an important area of needed research. Granulocytes represent an innate immune cell-type that generate cytokines and release their intracellular granules in response to foreign antigens. These cells are part of the innate immune response and the first line of defense for many organisms to protect from infection. We have previously shown that exposure of the rat basophilic leukemia (RBL) cell line to aqueous polyacrylic acid encapsulated metal-oxide nanoparticles (NPs) can dampen their degranulatory response at sub-lethal doses by interfering with the IgE antibody-Fcε receptor binding dynamics. In this study we examined the effects of NPs on cytokine secretion using a proteome profiler macroarray to detect the relative release of 40 different cytokines and chemokines. After 24h stimulation with IgE/DNP and PMA/ionomycin, inducible IL-3 and IL-4 levels were detected in addition to the constitutively secreted CXCL7, IL-13, sICAM-1, and VEGF. Therefore, we examined whether the secreted levels of the inducible and constitutive cytokines differ when exposed to NPs. RBLs were exposed to NP concentrations at previously identified lethal and sub-lethal doses over 24 hours. Activated cytokines were quantitatively examined using Western blot analysis. DLS characterization of NPs was also conducted at various exposure concentration and time points to determine the role aggregation plays in altering the cytokine secretory response.

**RP147 Effects of post-production disruption processes of graphene oxide nanoribbons on *Oryzias latipes* embryos and human cancer cell lines** S. Dasgupta, State University of New York at Stony Brook / School of Marine and Atmospheric Sciences; S. Mullick Chowdhury, B. Sitharaman, State University of New York at Stony Brook / Department of Bioengineering; A.E. McElroy, State University of New York at Stony Brook / School of Marine and Atmospheric Sciences. Carbon nanomaterials show great promise as molecular contrast agents and drug delivery vehicles. They are also used as components of building materials and a host of new thin film polymer applications. Hence the potential impacts of their release into the environment must be considered. The majority of toxicological assessments to date have focused on carbon nanomaterials functionalized with metals, such as silver, gold, or titanium oxide, while comparatively little is known about the toxicity of native nanomaterial scaffolds. Due to their insolubility in most aqueous media, high energy sonication steps are frequently used to create homogeneously dispersed stable suspensions of carbon nanomaterials in biological media. Experiments with embryos of Japanese medaka, *Oryzias latipes*, have demonstrated that exposure to probe-sonicated graphene oxide nanoribbon (GONR) solutions, even at very low concentrations (20µg/ml), elicited time-dependent increases in embryo toxicity. Such effects were absent in the low-energy bath sonicated GONR solutions. Parallel analyses of the effects of the sonicated GONR samples on human cancer cell lines (HeLa, A549 and MCF7 cells) demonstrated increased intracellular ROS production, and a time dependent decrease in cell metabolism, but no significant increases in cell death, indicating impacts at multiple levels of biological organization. Analysis using Raman spectroscopy indicated progressive structural changes associated with increased sonication time. These results indicate that the influence of post-production mechanical disruption should be considered to minimize the impacts of carbon-based



nanomaterial development. Ongoing research aims to further characterize the structural impact of sonication on GONR, the interaction between GONR and cellular components, and to elucidate mechanisms of toxicity.

**RP148 Expression of inflammatory cytokines in relation to intracellular ROS production in rat alveolar macrophages exposed to atmospheric particulate matter**

**D.S. Antkiewicz**, University of Wisconsin – Madison / Wisconsin State Laboratory of Hygiene, Wisconsin State Lab of Hygiene / Environmental Toxicology, University of Wisconsin – Madison / Wisconsin State Lab of Hygiene; **D.A. Perkins**, University of Wisconsin – Madison / Wisconsin State Laboratory of Hygiene; **Z. Sijan**, University of Wisconsin – Madison / Wisconsin State Lab of Hygiene; **M.M. Shafer**, University of Wisconsin-Madison / Environmental Chemistry & Technology, University of Wisconsin-Madison / Environmental Chemistry & Technology Dept.. Air pollution in the form of atmospheric particulate matter (PM) is a major concern in urban areas, which are home to approximately 50% of the world's population. PM has been linked to adverse human health effects such as asthma, bronchitis, respiratory infections, cardiovascular disease and cancer. Oxidative stress is an important toxicity pathway for PM exposures and the formation of excess Reactive Oxygen Species (ROS) can elicit an inflammatory reaction in pulmonary tissues. Alveolar macrophages are the primary cells that respond to both particulate and soluble portions of PM, producing ROS in the process and contributing to the inflammatory response. PM components that have been implicated in ROS formation include transition metals, soluble organic carbon and specific organic species, e.g. PAHs and quinones. However, our current understanding of the role and activity of different PM species in cellular oxidative stress is still very limited. Hence, new tools and approaches are needed to better characterize the toxicity pathways. To facilitate identification of the ROS active species as well as the mechanisms of ROS generation, we have employed an *in vitro* rat alveolar macrophage cell assay. Our method involves using a sensitive, broad spectrum fluorescent ROS probe (DCFH-DA) on both water extracts and suspensions of samples. PM samples were collected from contrasting urban environments to provide a range of real-world PM types. Additionally, we also obtained samples of selected primary PM source material. It has been shown that oxidative stress can lead to induction of pro-inflammatory cytokines such as TNF- $\alpha$ , IL-6 & IL-8. Moreover, some cytokines have been shown to trigger the release of other cytokines. To gain insight into the drivers of PM toxicity that lead to inflammation, RNA from the macrophages exposed to the various types of PM samples will be extracted and used with the Rat Inflammatory PCR Arrays (SA Biosystems) to assess relative expression levels of a broad suite of inflammatory cytokines and cytokine receptors. This, coupled with the ROS measurements and the knowledge of chemical composition of multiple types of PM will help us better understand the relationship between PM components and inflammatory response, as well as provide insights into cell signaling involved in the process.

**RP149 Food Chain Transfer of Quantum Dots from Primary Producer to Secondary Consumer**

**H.L. Freyaldenhoven**, / Ecotoxicology Research Facility; **J.L. Bouldin**, Arkansas State University / Department of Biological Sciences, Arkansas State University, Environmental Sciences Graduate Program / Environmental Sciences Graduate Program, Arkansas State University / Department of Environmental Science. Nanoparticles are increasingly being used in various industrial applications. Their ability to be highly specialized makes them ideal for usage in consumer products, biomedical diagnosis and treatment tools or any number of technologically advanced products. However with the recent exponential increase in the use and manufacturing of nanoparticles, current research is insufficient in demonstrating the environmental fate of these particles. The environmental fate of the nanoparticles is studied utilizing quantum dots (QDs; Qdot® 545 ITK™ Carboxyl Quantum Dots [Fisher Scientific; Invitrogen Molecular Probes, Eugene, OR, USA]) and their transfer from *Pseudokirchneriella subcapitata*, to *Ceriodaphnia dubia*, and then to *Pimephales promelas*. The concentration of cadmium from the QD core is determined utilizing atomic absorption spectrometry. These measured concentrations of cadmium are used as an indicator for potential transfer between each trophic level.

**RP150 How do nanoparticles affect cytochrome P-450-mediated Phase I detoxification processes of persistent organic pollutants?**

**Z. Lu**, University of Manitoba; **J. Veinot**, University of Alberta; **C.S. Wong**, University of Winnipeg / Richardson College for the Environment, Richardson College for the Environment. The influence of nanoparticles

on factors affecting cytochrome P-450 (CYP) isozyme-mediated phase I stereoselective biotransformation of chiral persistent organic pollutants (POPs) were investigated using a chiral polychlorinated biphenyl (PCB 95) and citrate-capped Au nanoparticles as probe compounds. Rat CYP 2B1 preferentially biotransformed the second-eluting atropisomer of PCBs 95 at low substrate concentrations ( $\leq 15 \mu\text{M}$ ). Competition with other substrates (including antipodal stereoisomers) decreased biotransformation rates stereoselectively, affecting atropisomeric composition. Homology modeling and docking studies suggested that individual PCB atropisomers had different interactions with rat CYP 2B1, and could dock with the isozyme at different locations. This is one possible explanation for stereoselective biotransformation and competition of chiral PCBs at the molecular level. Gold nanoparticles could disrupt the biotransformation activity of rat CYP 2B1 and change atropisomer composition of PCB 95 by electrostatic interactions among the isozymes, electrolytes, and substrates, thereby affecting the electron transfer chain in the CYP catalytic cycle. Depending on the concentration of gold nanoparticles, their presence could either enhance CYP activity through increasing regeneration of NADPH to drive the CYP catalytic cycle, or inhibit it by interacting with the CYP enzyme system directly. Our results suggest that the lack of predictive capability for stereoselectivity of PCBs and other chiral pollutants in biota may be due to competitive and/or inhibitory activities of different substrates which include individual isomers of the same compound, as well as potential interference from nanoparticles.

**RP151 Impacts of structure and functionalization of carbon nanomaterials on the toxicological and genomic response of the model organism *Daphnia magna***

**D.A. Arndt**, University of Wisconsin Milwaukee / Great Lakes Water Institute, University of Wisconsin Milwaukee / School of Freshwater Sciences; **R. Klaper**, University of Wisconsin-Milwaukee / School of Freshwater Sciences, University of Wisconsin-Milwaukee / Great Lakes Water Institute. Nano-toxicity literature indicates that nanomaterials can be toxic to organisms, however the mechanism of toxicity is still not well understood. Some studies show that oxidative stress is the primary mechanism of nano-toxicity, but it is possible that nanomaterials could also induce toxicity by other mechanisms. We examined how differences in core structure and surface chemistry affect the interaction of various carbon nanomaterials with the aquatic toxicological and genomic model species, *Daphnia magna*. *Daphnia* were exposed to sub-lethal concentrations of a variety of carbon nanomaterials that differed specifically in their core structure and surface functionality, and changes in the expression of genes involved in the organism stress response were measured. In addition, genes involved in other important physiological pathways were identified through next generation sequencing techniques, and their expression was measured across all exposure concentrations and nanomaterial types. Some pathways that were identified by 454 sequencing include pathways involved in metabolism, DNA damage, immune function, inflammation, and heat shock. Gene expression also varied with regards to different particle types, and this indicates potentially different mechanisms of action for distinct types of nanomaterials. This data demonstrates that nano-toxicity is more complex than just oxidative stress, and that nanomaterial core structure and surface functionalization can influence particle toxicity. In addition, there is a potential to use these expression patterns as molecular clues to determine nanomaterial exposures in aquatic environments.

**RP152 Long-term effects of ZnO nanoparticles on soil enzyme activities in unplanted and planted soils**

**S. Yoon, J. Kwak**, Konkuk University; **Y. An**, Konkuk University / Department of Environmental Science, Konkuk University / School of Earth and Environmental Sciences, Seoul National University / School of Earth and Environmental Sciences. This study evaluated the long-term effects of ZnO nanoparticles on the activities of six soil exoenzymes (dehydrogenase, fluorescein diacetate hydrolase, urease, acid phosphatase, arylsulfatase and  $\beta$ -glucosidase) in planted and unplanted OECD standard soils. Soil were treated with ZnO nanoparticles, and soil exoenzyme activities were measured at initial, day 0, day 28, and day 56. Among the enzymes tested, we observed that phosphatase activity was significantly inhibited throughout experiment period. There is a trend that activity of six exoenzymes is higher in unplanted groups than plant groups. *Acknowledgement. This subject is supported by Korea Ministry of Environment as the GAIA Project (2012000540011).*

**RP153 Metal oxide nanoparticles: Bioaccumulation and Trophic Transfer of Cerium in a Marine Bivalve** T.J. Baker, Exeter University / Biosciences; C. Tyler, University of Exeter / School of Biosciences; T.S. Galloway, University of Exeter / Biosciences Department. Metal and metal oxide nanomaterials (NPs: 1-100nm) are found in increasing use in industry, with particles such as cerium oxide, zinc oxide and silver heavily used in fuel, sunscreens and antimicrobial products respectively. Given the diverse shape and unique properties of NPs, this has led to a need for greater understanding of their ecotoxicological effects. Many authors have examined the toxicity of NPs to freshwater species, however the marine environment, and variables such as pH, temperature, salinity, particle coating and presence of organic matter, are less well studied. Chronic exposure to toxicants can also lead to bioaccumulation of that substance within the organism, which may then lead to trophic transfer to species higher in the food chain. In this experiment we present preliminary data examining the ability of cerium nanoparticles to bioaccumulate in the marine bivalve *Mytilus edulis* and, through consumption by higher invertebrates, examine their potential for transfer to other organisms in a simplified trophic food chain. The results of this experiment will contribute towards future policy decisions concerning concentration of nanoparticles found in wastewater releases.

**RP154 Mobility of Metal and Metal Oxide Nanoparticles in a Grenada Loring Series Field Soil** R.E. Boyd, US Army Engineer Research & Development Center / Environmental Laboratory; D.R. Johnson, U.S. Army Engineer Research and Development Center / Environmental Laboratory, U.S. Army / CEERD-EP-R, US Army Engineer Research and Development Center / Environmental Laboratory, US Army Engineer Research & Development Center / Environmental Laboratory; M.A. Chappell, US Army Engineer Research & Development Center; A.J. Bednar, US Army Engineer Research and Development Center / Environmental Laboratory; J.A. Stevens, US Army Engineer Research & Development Center. Recent advances in engineered nanotechnology have resulted in an exponential increase in the use of nanomaterials in everyday use items such as household cleaning products, laundry detergents, personal care products, pharmaceuticals and food products. These nanomaterials enhance the effectiveness and affordability of these products, but have the potential to end up in the terrestrial environment once they have reached the end of their life cycle. There are still many questions as to the fate and effects of these materials once they reside in the terrestrial environment. In order to answer some of these questions, we designed column soil leaching experiments with a local field soil with aged metal and metal oxide nanoparticles to determine whether natural aging will affect nanoparticle mobility. These experiments allowed us to look at the mobility of these particles which is directly related to their bioavailability and uptake. The Grenada Loring series soil (3% sand, 72% silt, and 26% clay, 0.7% total organic content with 1% loss upon ignition) was collected from the Brown Loam Experiment station (Raymond, MS), dried, milled, and sieved through a #10 screen. The target concentration of the metal and metal oxide nanoparticles (TiO<sub>2</sub>, ZnO, CeO, Citrate-Ag, and PVP-Ag) in the soil was 100 mg/kg. The soils were allowed to age for over 18 months in an enclosed container. The columns (6.7cm W x 50.3 cm H) were packed with the aged soils, saturated with a 0.5 mM CaCl<sub>2</sub> solution, allowed to acclimate for 4 hrs, then leached for 72 hrs. Each nanoparticle experiment was run in duplicate while collecting leachate samples every hour with an autosampler. These leachate samples were analyzed for pH, EC, and total organic carbon. Total dissolved and particulate metals were analyzed by inductively coupled plasma (ICP)-mass spectrometry or ICP-atomic emission spectroscopy. The leached soil was sampled at 4 different evenly distributed depths. The experiments will provide valuable insight into terrestrial mobility of aged nanoparticles, especially when terrestrial applications of nanoparticles (e.g., nano-pesticides) increase as well as increased disposal of consumer products containing nanotechnologies.

**RP155 Nanoparticle-dermal surface interaction on *Caenorhabditis elegans*** S. Kim, Konkuk University; Y. An, Konkuk University / Department of Environmental Science, Konkuk University / School of Earth and Environmental Sciences, Seoul National University / School of Earth and Environmental Sciences. This study highlights the interaction of metal nanoparticles with dermal surfaces of the nematode *Caenorhabditis elegans*. The test nanoparticles are silver (AgNPs), titanium dioxide (TiO<sub>2</sub>), and zinc oxide (ZnO) nanoparticles. The survival of *Caenorhabditis elegans* evidenced a clear decrease in nanoparticle amended growth media. NOECs of reproduction were determined to be 1 mg/L (AgNPs), 100 mg/L (TiO<sub>2</sub> NPs),

and 10 mg/L (ZnO NPs), respectively. We noted the significant interactions of all test nanoparticles with the skin of *Caenorhabditis elegans*. Severe epidermic edema and burst were observed in the exposure group, which may be related with infections in soil environments. *This work was supported by the National Research Foundation Grant funded by the Korean Government (NRF 2011-0015985).*

**RP156 Preferential Sorption of Natural Organic Matter to Nanoparticles: Influence of pH and Ionic Strength** P. Mwaanga, Clemson University / Environmental Toxicology; E. Carraway, Clemson University / Environmental Engineering & Earth Sciences, Clemson Institute of Environmental Toxicology. Natural organic matter (NOM) is ubiquitous in the aquatic environment and it plays a great role through its interactions affecting transport of various chemicals and in nutrient cycling. NOM is involved in a number of mechanisms responsible for complexation, reduction, mobilization or immobilization of toxicants and hence in modulating bioavailability. However, different NOM size fractions, depending on their molecular size and conformations in different environmental conditions may enhance or mitigate toxicity. Therefore, understanding the changes or fractionation of NOM size upon sorption to nanoparticles may help improve our predictive capabilities on the behavior of NOM and nanomaterials in environment. In this study, the fractionation of NOM upon sorption to titanium (TiO<sub>2</sub>) nanoparticles was examined. The study specifically examined the influence of pH, ionic strength and NOM concentration on the extent of fractionation at a constant sorbent concentration (TiO<sub>2</sub>). High performance size exclusion chromatography (HPSEC) was used to study the changes in the molecular sizes of NOM. Corroborative evidence was obtained from optical techniques such as absorbance and fluorescence spectrophotometry. The total organic carbon was measured by the Total Organic Carbon Analyzer- Shimadzu (TOC-VCPh). The results indicated that fractionation of NOM occurs upon sorption to TiO<sub>2</sub> nanoparticles irrespective of NOM concentration. However, at any NOM concentration, the greatest fractionation was observed at lower pH and at higher ionic strength. Fractionation decreased with increasing pH and decreasing ionic strength over the range of 7.5 mg C/L to 15 mg C/L NOM concentration used in this study. Both absorbance and fluorescence spectrophotometry data gave credible corroborative evidence on the extent of fractionation with respect to pH, ionic strength and NOM concentration.

**RP157 Promotion of immune responses by pretreatment silica nanoparticles in mice** K. Higashisaka, Y. Yoshioka, T. Nagano, A. Kunieda, K. Hata, Graduate School of Pharmaceutical Sciences, Osaka University / Laboratory of Toxicology and Safety Science; S. Tsunoda, National Institute of Biomedical Innovation (NiBio) / Laboratory of Biopharmaceutical Research (LBR); H. Nabeshi, National Institute of Health Science; T. Yoshikawa, Y. Tsutsumi, Graduate School of Pharmaceutical Sciences, Osaka University / Laboratory of Toxicology and Safety Science. Summary: With the recent development of the nanotechnology, nanomaterials have been rapidly spreading to a wide variety of fields such as cosmetics and food technology. In particular, silica nanoparticles possess extraordinary advantages, including straightforward synthesis, relatively low cost, and easy surface modification. Therefore, we cannot help avoiding intake of silica nanoparticles through any kind of exposure pathway in our life. On the other hand, it is concerned that nanomaterials might have potential risks attributed to their unique properties on biology and environment. Numerous studies have shown that several types of nanomaterials have possibility to increase the incidence of immune diseases. However, the role of the different physical characteristics of nanomaterials in the production of immune responses has not been elucidated. Here, we examined the relationship between the size of silica particles and immune responses. Female BALB/c mice were intravenously treated with silica particles with diameter of 70 or 1000 nm (designated nSP70 and mSP1000, respectively) at concentrations of 0.8 mg/mouse on day 0. The next day, mice were intraperitoneal exposed to ovalbumin (OVA) as antigen or OVA adsorbed on Imject Alum (alum). On day 15, plasma was collected to assess OVA-specific antibody responses. OVA-specific IgG and subclass IgG1 antibody levels were determined by enzyme-linked immunosorbent assay (ELISA). ELISA analysis showed that the levels of anti-OVA IgG antibody and anti-OVA IgG1 antibody induced by OVA plus nSP70 were tend to increase in comparison with those induced by OVA alone or OVA plus mSP1000. In addition, OVA-specific antibody responses in mice exposed to OVA plus nSP70 was higher than those exposed by OVA adsorbed alum as positive control. These results suggest that silica nanoparticles can induce the



production of OVA-specific antibody responses including antigen-specific Th2 allergic immune responses. Although it is not clear why silica particles would induce Th2-polarized allergic immunity, we are now trying to analyze the profiles of cytokines in OVA-exposed mice to clarify the mechanism. Taken together, we consider that further studies of the relationship between the characteristics of nanomaterials and immune responses will facilitate the development of safe and effective nanomaterials.

**RP158 Short Term Toxicity of TiO<sub>2</sub>, Al<sub>2</sub>O<sub>3</sub> and CeO<sub>2</sub> Nanoparticles to *E. coli* Bacteria under Ambient Conditions** A. Erdem, Akdeniz University / Environmental Engineering, Akdeniz University / Dept. of environmental engineering; M. Ozkaleli, Akdeniz University / Dept. of environmental engineering; N.A. Perendeci, Akdeniz University / Department Environmental Eng.. Nanotechnology deals with nanomaterials, which is in the range of 0.1 to 100 nm, to understand their novel properties, to increase their controllability and to improve the processes using this technology. Currently, the contribution of nanotechnology to the world economy is unquestionable. Nanotechnology is actively being pursued in many production and manufacturing sectors. Among the various nanotechnology products, metal oxide nanoparticles (NPs) have being the most used nanomaterials in industry. However, the rapid increase in the production and the extensive usage of the NPs bring about many environmental problems. The adverse effects of NPs on human and environmental health remain questionable. There are still limited scientific studies on the affects of NPs, and there is no environmental standard and implementations on the NPs, yet. Moreover, the main mechanism of the inactivation of aquatic organisms, the roles of the NPs in the inactivation of those organisms, the factors and properties of the NPs affecting the ecotoxicity are still remain unknown. Especially, there is a lack of studies on the ecotoxicity of manufactured metal oxide NPs in the literature. The effects of some metal oxide NPs on the survival or die-off of *E. coli* (ATCC 25912) were investigated under ambient conditions. Experimentally, a 16-h *E. coli* cultures were exposed to TiO<sub>2</sub>, Al<sub>2</sub>O<sub>3</sub> and CeO<sub>2</sub> NPs at various concentrations and pH's both in darkness and the presence of a simulated solar light. Preliminary results indicated that *E. coli* underwent a two-stage response to NPs independent of UV light irradiation; a rapid decrease in population density within the first 10 min then slowly decreased upon extended treatment time, e.g., 30-60 min. Results also show that there is bacteria die-off in the presence of nanoscale TiO<sub>2</sub> in dark. Generally it appears that the nano-TiO<sub>2</sub> toxicity increases as the particle size decreases. The presence of light irradiation significantly enhanced the inactivation of the bacteria due to additional photocatalytic activity of TiO<sub>2</sub>. Photocatalytic reactions generated hydroxyl radical, which is strong oxidation agent that can cause great stress and damage to the cell. Cell damage was also observed in terms of lipid peroxidation (e.g., production of malondialdehyde, MDA). Upon exposure of *E. coli* to NPs the photocatalytic activity that was generated has markedly increased the production of MDA.

**RP159 Single-walled carbon nanotubes decrease the toxicity and bioaccumulation of PCBs in marine benthic invertebrates exposed to contaminated sediment** A.N Parks, Duke University / Nicholas School of the Environment; L.M. Portis, J. Sullivan, U.S. EPA; R.M. Burgess, U.S. EPA / Atlantic Ecology Division, National Health and Environmental Effects Research Laboratory, U.S. EPA / Ord/Nheerl-Atlantic Ecology Div; K.T. Ho, U.S. EPA / Atlantic Ecology Division; M.M. Perron, NRC/ EPA; G. Chandler, University of South Carolina / Department of Environmental Health Sciences, University of South Carolina / Arnold School of Public Health; L. Ferguson, Duke University / Department of Civil and Environmental Engineering, Duke University / Nicholas School of the Environment, Pratt School of Engineering / Department of Civil & Environmental Engineering. As their use increases in consumer products, nanoparticulate contaminants such as single-walled carbon nanotubes (SWNT) may start to occur in aquatic environments with organic contaminants such as hydrophobic organic chemicals (HOCs). In the case of contaminated sediments from New Bedford Harbor (NBH, MA, USA), the dominant organic pollutant is polychlorinated biphenyls (PCBs). Some of the most dominant congeners include CB-18 (96.4 µg/g), CB-28 (224.2 µg/g), CB-52 (121.8 µg/g), CB-110 (74.0 µg/g), and CB-118 (84.9 µg/g). Previous work in our laboratory has shown that polycyclic aromatic hydrocarbon (PAH) bioavailability to marine benthic organisms is altered when SWNT are present due to their sorption to the surface of SWNT. To investigate this phenomenon further in environmentally relevant sediment, we made dilutions of NBH sediment (0%, 25%, 50%, 75%, and 100%) with

a non-contaminated marine sediment. Sediments were then amended at 1 mg/g and 10 mg/g with pristine SWNT and the activated carbon coconut charcoal (CC). Our objective was to determine if the toxicity and bioaccumulation of the PCB contaminants in a benthic amphipod (*Ampelisca abdita*), mysid (*Americamysis bahia*), and polychaete (*Nereis virens*) would be mitigated by the presence of SWNT and if the impact would differ from that of CC. The results of our work show increased survival in treatments amended with SWNT but not CC suggesting a SWNT-specific effect on the bioavailability of PCBs under the conditions evaluated. For example, in the 25% NBH treatment, survival of *A. bahia* and *A. abdita* both increased by approximately 20%. Further, the presence of SWNT in the 50% NBH treatment allowed 50% survival of *A. bahia* compared to 0% in the sediment treatment without SWNT. The presence of SWNT at 10mg/g also led to an overall decrease in PCB bioaccumulation by the polychaete in the 25% NBH treatment. Specifically, total PCB concentrations were decreased by a factor of three, and a few low molecular weight congeners (e.g., CB-18 and CB-28) were reduced by a factor of four. These results suggest that SWNT, when present in the environment, will act as a strong sorbent for HOCs, thereby reducing the bioavailability, bioaccumulation and toxic impact of these contaminants.

**RP160 The Effects of CuO an Al<sub>2</sub>O<sub>3</sub> Nanoparticles on Waste Activated Sludge Anaerobic Digestion** E. KokdemirUnsar, Akdeniz University; A. Erdem, Akdeniz University / Environmental Engineering, Akdeniz University / Dept. of environmental engineering; N.A. Perendeci, Akdeniz University / Department Environmental Eng.. The rapid development of nanotechnology accelerates the use of nanoparticles in many industrial products, such as antibacterial coatings, catalysts, biomedicine, and personal care products, because of their unique magnetic, electrical and optical physicochemical properties. With the world wide utilization of nanoparticles, increasing attention has been paid to potential effects of nanoparticles on environment. Most studies have focused on the toxicity of nanoparticles to human health, soil and aquatic organisms. Intentionally or unintentionally released nanoparticles finally ends up in the wastewater collection systems and then enters the wastewater treatment plants. The existence of nanoparticles in wastewater treatment plants has been reported and the adsorption, aggregation and settling of nanoparticles by activated sludge has been reported as the main removal mechanism in wastewater treatment plant. Large amounts of waste activated sludge are produced in wastewater treatment plants and different sludge removal processes are used to stabilize the sludge before discharging into the environment. The anaerobic digestion of waste activated sludge is a widely preferred option, since both energy recovery via methane generation and final disposal of activated sludge have been achieved. But, limited number of papers has been addressed to the effects of nanoparticles on anaerobic digestion. In this study, the effects of CuO an Al<sub>2</sub>O<sub>3</sub> nanoparticles on methane production during anaerobic digestion of sewage sludge were evaluated and the EC50 concentrations of CuO an Al<sub>2</sub>O<sub>3</sub> nanoparticles on anaerobic consortium were determined. For this purpose, sewage sludge taken from Antalya wastewater treatment plant were characterized in terms of pH, TSS, VSS, sCOD, tCOD, TKN, total protein and total carbohydrate. The purchased CuO and Al<sub>2</sub>O<sub>3</sub> nanoparticles are also characterized with the measurement of particle size, zeta potential, and SEM analysis. The effects of CuO an Al<sub>2</sub>O<sub>3</sub> nanoparticles on methane production and EC50 concentrations of CuO an Al<sub>2</sub>O<sub>3</sub> nanoparticles on anaerobic consortium were performed by the standart biochemical methane potential (BMP) test, and the inhibition of gas production of anaerobic bacteria test (ISO 13642-2), respectively. CuO nanoparticles are identified toxic particles to anaerobic bacteria and high concentration of CuO nanoparticles inhibit the methane generation of anaerobic digestion.

**RP161 The Influence of Wastewater on Gold Nanoparticle Physicochemical Characteristics and Subsequent Accumulation in *Daphnia magna*** A.T Wray, Clemson University / Institute of Environmental Toxicology, Clemson University; S.J. Klaine, Clemson University / Institute of Environmental Toxicology (CU-ENTOX), Clemson Institute of Environmental Toxicology / Institute of Environmental Toxicology (ENTOX), Clemson University / Institute of Environmental Toxicology (ENTOX). Environmental exposures from nanomaterials will become increasingly prevalent as private and public financing bolsters reinvention of industrial progress through nanotechnology endeavors. The journey from consumer use to aqueous release is particularly relevant to nano-toxicology because this journey will define the physicochemical characteristics of the nanomaterial



that could have implications on the behavior and fate within the receiving ecosystem. Nanomaterials released from consumer and industrial products are likely to face the gauntlet of a wastewater treatment facility wherein an individual particle will be open to a number of interactions with both the wastewater components and activated sludge modifying the particle prior release into an aquatic ecosystem. The aim of our research is to define how these interactions will affect the downstream particle-biota relationship. Specifically we are interested in simulating the effects of wastewater incubation on waterborne gold nanoparticle uptake and elimination kinetics in *Daphnia magna*. We utilized several particle configurations that contained either a positive, negative, neutral (control) or zwitterionic surface charge and separated the exposures into two treatments: unadulterated nanoparticles and adulterated nanoparticles that were incubated for 24 hours with synthetic wastewater. The exposure solution with adulterated nanoparticles was intended to simulate interactions between nanoparticles and wastewater components that would occur in the water column as the particles advance through the wastewater treatment plant. Elimination rate constants were derived for each particle configuration to predict bioaccumulation potential. Overall, we observed differential elimination based on initial particle surface charge and a noticeable shift in patterns after exposure to wastewater. Based on the known components of wastewater, the formation of a protein corona is the likely mechanism facilitating the differential accumulation patterns noted between treatments.

**RP162 The Mitigative Influence of Natural Organic Matter on Toxicity of Zinc and Copper Oxide Nanoparticles to *Daphnia magna*** P. Mwaanga, Clemson University / Environmental Toxicology; P. van den Hurk, Clemson University / Biological Sciences; E. Carraway, Clemson University / Environmental Engineering & Earth Sciences, Clemson Institute of Environmental Toxicology. Prior research has shown that natural organic matter (NOM) and ionic strength have great influence on the aggregation and dissolution characteristics of metal oxide nanoparticles. Once aggregated or dissolved, the toxicology of metal oxide nanoparticles may be modified. Research is still ongoing into investigating the extent to which NOM and ionic strength influence nanoparticle toxicity. Knowledge to be gained from such research will help in elucidating mechanisms of toxicity mitigation. As part of the ongoing research, this study assessed the influence of NOM on the toxicity of two metal oxide nanoparticles (nZnO and nCuO). The study examined the influence of NOM toxicity of these metal oxide nanoparticles at two levels of biological organization; organism (acute toxicity) and cellular (biomarkers) levels. The assessments at organism level were conducted in different test media (soft water, moderately hard water, FETAX solution). The assessments at cellular level were conducted in moderately hard water only. *Daphnia magna* was used as an assessment organism in this study. Organism effects were monitored through measuring mortality after 48 h exposure and cellular effects were monitored by measuring a select suite of biomarkers such as glutathione-S-transferases (GST), thiobarbituric acid reacting substances (TBARS), oxidized glutathione (GSH) and metallothionein (MT) after 72 h of exposure to sublethal concentrations. The results indicated that both metal oxide nanoparticles are toxic to *Daphnia magna*. The 48 h LC<sub>50</sub>s were highest in FETAX solution and lowest in soft water, indicating reduced toxicity in FETAX solution. In the presence of dissolved NOM even at 1 mg/L, the toxicity was dramatically reduced in soft water and moderately hard water. While in FETAX solution there was virtually no toxicity observed for the range of concentrations used in this study. For the cellular effects, both nanoparticles showed inhibition of GST, increased levels of malondialdehyde (MDA) measured as TBARS, increased oxidized GSH and induction of metallothionein. In the presence of NOM, these effects were less pronounced. Overall the results suggest NOM has a mitigative effect on metal oxide nanoparticle toxicity.

**RP163 Toxicity assessment of Titanium Dioxide and Cerium Oxide nanoparticles in *Arabidopsis thaliana*** L. L. Tumburu, National Research Council, US Environmental Protection Agency; P.T. Rygielwicz, US EPA NHEERL/WED / Research Ecologist; J. Betts, Dynamac Corporation; C.P. Andersen, US EPA NHEERL/WED / Plant Physiologist; M.G. Johnson, U.S. Environmental Protection Agency / National Health and Environmental Effects Research Laboratory; G. King, Dynamac Corporation; J.R. Reichman, US EPA NHEERL/WED; M. Plocher, M. Storm, Dynamac Corporation. The production and applications of nanoparticles (NP) in diverse fields has steadily increased in recent decades; however, knowledge about risks of NP to human health and ecosystems is still scarce. In this

study, we assessed potential toxicity of two commercially used engineered nanoparticles (ENP), titanium dioxide (TiO<sub>2</sub>) and cerium oxide (CeO), using thale cress *Arabidopsis thaliana* as a model system. The responses of the plant to ENPs, at concentration 500mg/L and ~100nm diameter range, were observed at morphological, physiological and molecular genetic levels. ENP exposed *Arabidopsis* seedlings showed enhanced germination (radical emergence, cotyledon and hypocotyl visibility, fully grown leaves) at different growth stages. Phenological observations revealed initial differences in plant growth (number of rosettes, rosette diameter, and plant height) between the treatment and the control. However, this difference was not noticed in the later stages of development. For molecular studies, *Arabidopsis* plants were exposed to the ENPs, starting in the seed stage, and subsequently with the growth of plants, both root and shoot systems were also exposed. Three different tissues (cotyledons, roots, and leaves) at different developmental stages were harvested for transcriptomic analysis using microarrays. RNA was extracted from the harvested tissues, processed into biotin-labeled cRNA and hybridized to Affymetrix GeneChip *Arabidopsis* ATH1 Genome Arrays (Affymetrix) to obtain the tissue-specific transcriptome profile of *Arabidopsis*. We identified several functional pathways including oxidative stress pathways that were affected by exposure to TiO<sub>2</sub> and CeO<sub>2</sub> nanoparticles. Our findings so far convey that the two ENPs in this study have the potential to affect initial growth and development of the plant (germination stages), as well as affecting various signaling pathways at the transcriptome level in tissue-specific pattern. Although our observations did not reveal any significant differences at the later growth stages of *Arabidopsis*, further studies are required to evaluate if toxicity associated with these ENPs can alter plant response to other environmental stresses, or whether ENP effects may be passed on transgenerationally to subsequent progeny.

**RP164 Toxicity of ZnO Nanoparticles to the Copepod, *Acartia tonsa*, Exposed via a Phytoplankton Diet** T.A. Jarvis, Valdosta State University / Department of Biology; R. Miller, H. Lenihan, University of California, Santa Barbara; G. Bielmyer, Valdosta State University / Department of Biology. Zinc oxide (ZnO) nanoparticles are being increasingly utilized in a variety of products and applications and are therefore more commonly discharged into aquatic environments. Increased production and use of these manufactured ZnO nanoparticles may increase exposure and pose a toxicological threat to aquatic organisms. In this study, the diatom, *Thalassiosira weissflogii* was exposed to ZnO for 7 d and growth, zinc accumulation and cellular zinc distribution within the algal cells were measured. Additionally, the toxic effects of dietary ZnO exposure were determined. *T. weissflogii* cultured with ZnO was continuously fed to the marine copepod, *Acartia tonsa* for 7 d and reproduction and survival were quantified. A dose-dependent growth reduction was observed in *T. weissflogii* exposed to ZnO, with a 20% effect concentration (EC20) of 24 mg/L and a LOEC of 10 mg/L. Zinc accumulation in the algae occurred dose-dependently over time with the majority of the zinc partitioning into the cell wall fraction. A decrease in copepod survival and reproduction was also observed after copepods were fed the ZnO-laden diet. The EC20s were 112 mg/L and 62 mg/L and LOECs were 168 mg/L and 263 mg/L for copepod survival and reproduction, respectively. The values correspond to the zinc concentration in the *T. weissflogii* exposure media. These results provide new data concerning the effects of ZnO in a sensitive marine phytoplankton-zooplankton food chain.

**RP165 Uptake, subcellular distribution and toxicity of nano-CuO, micron-CuO and aqueous Cu in the benthic polychaete, *Nereis diversicolor*** A. Thir, Roskilde University / Environmental, Social and Spatial Change; G. Banta, H. Selck, Roskilde University. Engineered metal nanoparticles (NPs), such as Copper (Cu) NPs are increasingly being used in consumer products, and human and environmental exposure is likely to increase. Cu is an essential metal as it serves as a co-factor for a variety of enzymes (Zhou and Gitschier 1997). However, Cu is highly toxic in excess due to its ability to bind to proteins inhibiting their activity, to interfere with homeostasis of other metals, to generate free radicals, and to damage DNA. Cu NPs may cause adverse effects and potentially be toxic, as a result of their small size (< 100 nm (BSI 2007)) and high surface to volume ratio. Engineered metal NPs have been shown to cause organelle damage, DNA-damage, oxidative stress, apoptosis, up/down regulation of proteins etc. Before CuO NPs can cause potential toxic effects to the organism, the particles must be accessible to the organism and able reach the site of action (e.g., proteins and DNA) either directly or indirectly via the release of ions or production of reactive oxygen species. In this study we examine dietary uptake, intracellular fate

and toxicity of Cu in different forms and particle sizes in the sediment-dwelling *Nereis diversicolor*. The benthic environment is a likely exposure scenario as it serves as a sink for many hydrophobic contaminants. Cu NPs released into the aquatic environment will likely agglomerate and/or aggregate upon contact in the brackish water of the estuary and subsequently accumulate in the sediment compartment. The present study examines uptake, subcellular distribution and toxicity of sediment associated Cu of different forms and particle sizes in *Nereis diversicolor*. This is addressed by comparing effects of aqueous Cu (administered as  $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ ) and CuO particles of different sizes; NPs (polydispersed-100 nm) and micro particles (5  $\mu\text{m}$ ) after 10 days of exposure.

**RP166 Use of a Comprehensive Toxicological Suite for the Analysis of Airborne Particulate Matter** J. Turner, University of Colorado at Boulder / Civil, Environmental and Architectural Engineering; K. McCabe, University of Colorado at Boulder / Multicellular and Developmental Biology; M.T. Hernandez, University of Colorado at Boulder / Civil, Environmental and Architectural Engineering. Epidemiological studies have provided compelling statistical evidence for associations between atmospheric particulate matter (PM) exposures and negative health effects. Using bacterial and mammalian cell lines, numerous studies have provided converging lines of mechanistic evidence to demonstrate that beyond certain thresholds, airborne PM has the potential to introduce DNA damage, cytotoxicity and inflammation via oxidative stresses. The US EPA now regulates daily mass averages of coarse and fine airborne PM levels under the Clean Air Act. Because the biochemical and toxicological properties of PM are not considered, these modern ambient air quality standards are generalized, lacking practical understanding of the negative biological activity PM can convey from sources to receptors. Recent toxicology research in the atmospheric environment focuses primarily on inflammatory biomarkers, which generalize cellular damage pathways to a solitary mechanism. Such an approach limits more robust perspectives needed to comprehensively describe the conglomerate of biological activities PM may carry. In response to these limitations, a suite of single-platform cytometric toxicological assays, employing human cell lines (lung epithelial and phagocytic monocytes), has been adapted to simultaneously assess major toxicological modes associating with airborne particulate matter *in-situ*. Induction has high sensitivity across this assay panel, which implicates the following classes of fundamental cellular responses previously linked with PM exposure: (i) necrotic and apoptotic cytotoxicity, (ii) genotoxicity and (iii) oxidative stress. These assays are relatively rapid and can provide concomitant indications of individual toxicity mechanisms, as well as present the potential for synergistic contributions to the negative health effects PM can convey. This approach may serve as a field adaptable template for mechanistic-based PM regulations.

**RP167 Using comprehensive machine learning approach to predict submicron air pollution indicators** L. Jian, Curtin University / Research Fellow, Curtin University / School of Public Health; G. Pandey, Mount Sinai School of Medicine. The regulation of air pollutant levels is rapidly becoming one of the most important tasks for the governments of developing countries, especially China. However, the relationship between concentration of atmospheric submicron particles, such as ultrafine particles (UFP) and particulate matter  $\leq 1.0$  micrometers (PM<sub>1.0</sub>), meteorological and traffic factors is poorly understood. In this study, we employed a range of advanced machine learning techniques to predict UFP and PM<sub>1.0</sub> levels based on a data set consisting of observations of weather and traffic variables recorded at a busy roadside in Hangzhou, China. Based upon the thorough examination of over twenty five classifiers implemented for this task, we find that it is possible to predict PM<sub>1.0</sub> and UFP levels accurately and these tree-based classification models (Alternating Decision Tree and Random Forests) perform the best for both PM<sub>1.0</sub> and UFP. This study has demonstrated the promising value of systematically collecting and analysing data sets using machine learning techniques for prediction of submicron sized ambient air pollutants.

**RP168 ZnO Nanomaterials Induce Apoptosis in Sea Urchin Embryos in a Stage-Specific Manner** E.A. Fairbairn, UC Davis / Bodega Marine Lab, University of California--Davis / Bodega Marine Laboratory; G.N. Cherr, University of California-Davis / Bodega Marine Laboratory. The impact of nanomaterials (NMs) on early life stages of marine organisms is an important question in ecotoxicology that has received little attention. Metal oxide nanomaterials (e.g. ZnO NM) are widely used in consumer products,

including personal care products such as sunscreen. Their toxicity has often been attributed to dissolution in aqueous media with the free metal ions resulting in cytotoxicity. Our research has shown that ZnO NM is highly toxic (as low as 50  $\mu\text{g/L}$ ) to developing sea urchin embryos (Fairbairn et al., 2011, J. Haz. Mat.). By examining the solubility of ZnO NM, bulk ZnO and soluble  $\text{Zn}^{2+}$  in seawater, and comparing their toxicities in developing sea urchin embryos, we were able to attribute the toxicity of ZnO NM in seawater to soluble  $\text{Zn}^{2+}$ . Using two assays for apoptosis (caspase and TUNEL) we now demonstrate that ZnO NM can induce apoptosis in cells of developing sea urchin embryos. The ability of ZnO NM to induce an increase in the number of apoptotic cells per embryo is stage specific: the blastula and gastrula stages show increased apoptosis with ZnO NM exposure, while the pluteus stage does not.

**RP169 Acute Toxicity of Cypermethrin on *Oreochromis niloticus* and *Clarias gariepinus*** E. Nestor, University of Dschang / Department of Animal Biology, University of Dschang. Insecticides, particularly pyrethroid insecticides, form the bulk of pesticides used in Cameroon. More and more vegetable farmers are using these pesticides and since their activities usually take place in the immediate proximity of freshwater bodies, the risk of contamination of these water bodies and causing undesired effects is high. A series of acute static short term bioassays were carried out in this research project to assess the effect of Cypermethrin, a synthetic pyrethroid insecticide, on the fingerlings of two local fish species: *Oreochromis niloticus* and *Clarias gariepinus*. Entire dose-response relationships were established and the concentration resulting in 50% mortality (LC<sub>50</sub>) calculated for different exposure times (24, 48, 72 and 96 h) for each of the two species. We obtained LC<sub>50</sub>s that ranged from 23 to 24.5  $\mu\text{g/L}$  for *Oreochromis niloticus* and 12.4 to 14.98  $\mu\text{g/L}$  for *Clarias gariepinus*. This shows that Cypermethrin is highly toxic to both fish species with *Clarias gariepinus* being more sensitive. We also observed behavioural responses such as irregular, erratic and darting swimming movements as well as escaping behaviours in which the fingerlings attempted to jump out of the medium containing Cypermethrin. In addition, other behaviours such as increased frequenting of the water surface were observed all of which were sensitive indicators of the effect of Cypermethrin. We strongly recommend that this insecticide be used with caution only by trained farmers in order to prevent contamination of our aquatic ecosystems.

**RP170 Age and temporal, effects of pyriproxyfen on fecundity and sex determination in *Daphnia magna*** G.K. Ginjupalli, Clemson University / PHD student, Institute of Environmental Toxicology / PHD student; W.S. Baldwin, Institute of Environmental Toxicology / Department of Biological Sciences. Pyriproxyfen is an insecticidal juvenile hormone analog that perturbs insect development and is therefore used to control a variety of insects and pests. Pyriproxyfen also perturbs normal parthenogenic (clonal) reproduction non-target cladoceran species because it induces male production that can lead to a decrease in fecundity and a reduction in population density. Consequently algal blooms may occur, and the food web may be disturbed by the loss of arthropods leading to starvation by higher level consumers. In this study, we investigated the impact of pyriproxyfen on *Daphnia magna* reproduction using a modified chronic toxicity tests. Daphnids at or near reproductive age were exposed to pyriproxyfen for approximately 10-12 days or until they had 4 broods, and the number and sex of the offspring was determined. Pyriproxyfen reduces overall fecundity (production of males + females) and increases male production in a concentration-dependent fashion (0.82 to 13.12 picomolar). In addition, older *Daphnia* (21 + days of age) produce males faster (in large numbers by the 2<sup>nd</sup> brood), but show refractory effects as the older *Daphnia* produce less males by the 4<sup>th</sup> brood compared to younger *Daphnia* (7 + days of age). This suggests a potential mechanism by which healthy populations that includes all ages of *Daphnia* may be less susceptible to pyriproxyfen's effects but still responsive to environmental stressors that require male production. In addition, short-term or episodic exposures at 1.64 and 3.2 picomolar concentrations also induced male production. Interestingly, the length of exposure is not crucial in causing the initial male production; however, continued exposure causes extended male production. Taken together, use of pyriproxyfen around water bodies needs due caution because of the potential adverse effects on *Daphnia* reproduction regardless of whether the exposure is for short or long periods of time.

**RP171 Agricultural chemicals in surface waters and sediments from the Upper Conasauga River in northwest Georgia, USA** P.J. Lasier, USGS-Patuxent Wildlife Research Ctr. / University of Georgia; M.L. Ulrich, USGS-Patuxent Wildlife Research Ctr; S.M. Hassan, University of Georgia; P.D. Hazelton, University of Georgia / Warnell School of Forestry & Natural Resources, University of Georgia / graduate research assistant; R.B. Bringolf, University of Georgia / Interdisciplinary Toxicology; K.M. Owens, The Nature Conservancy; E. Prowell, U. S. Fish and Wildlife Service. Hormones, glyphosate, nutrients, and metals were investigated as potential causes for fish and mussel declines in the Conasauga River. Surface-water samples were collected from the mainstem, major tributaries, and agricultural ditches after major rain events. Sediments were collected above and below the tributaries in mid-summer. *Ceriodaphnia dubia*, *Hyalella azteca* and *Lampsilis siliquoidea* were used to assess water and sediment toxicity. Sediment-estrogen levels were variable, but elevated downstream of a dairy farm (0.38 mg estradiol/kg, dw) and the most suburban tributary (3.2 mg estrone/kg). Glyphosate was rarely observed in water samples, but its primary degradation product, aminomethyl phosphonic acid (AMPA), was detected in 77% of the samples. AMPA concentrations ranged up to 5.7 mg/L among samples from the ditches and up to 0.15 mg/L in mainstem and tributary samples. AMPA was non-detectable in most ditch samples after the farmer changed from tank mix to a commercial product (Round-up®). Most sediment samples contained glyphosate and AMPA, but AMPA was usually present in much greater concentrations ( $\leq 2.4$  mg/kg). Occasionally, spikes of nitrate and phosphate were observed in surface waters, but water- and sediment-metal concentrations were relatively low. Few water samples indicated chronic toxicity. However, sediment toxicity occurred at the two sites where estrogen concentrations were greatest; although inconsistent results may indicate other causes. *Hyalella azteca* growth was reduced in sediments from both sites, but *L. siliquoidea* growth decreased in one and increased in the other. Estrogens and glyphosate/AMPA were pervasive in these sediments indicating a need to understand their long-term effects.

**RP172 An active biomonitoring approach to assess pesticide exposure from an irrigation scheme in a semi arid region of South Africa** W. Malherbe, Centre for Aquatic Research, University of Johannesburg / Zoology Department, Centre for Aquatic Research, University of Johannesburg / Department of Zoology; V. Wepener, Centre for Aquatic Research, University of Johannesburg / Zoology, Rand Afrikaans University; J. VAN VUREN, Centre for Aquatic Research, University of Johannesburg. The aquatic environment is subjected to multiple stressors and pollutants that potentially have detrimental effects on the aquatic organisms either directly or due to interactions with other stressors or environmental factors. Active biomonitoring has been proven to be an effective tool to identify toxic effects in aquatic systems when stressors are unknown or multiple stressors are present. *Tilapia sparrmanii* was collected at a relative reference site located upstream of a large scale irrigation scheme in a semi-arid area of South Africa. The collected fish were then transplanted at two localities receiving irrigation return flows, waste water and numerous other sources of pollutants. The biomarker analysis included a biomarker of exposure, acetylcholinesterase (AChE), and biomarkers of effects, malondialdehyde (MDA), catalase (CAT) activity and protein carbonyls (PC). These biomarkers are all indicative of oxidative stress that can be caused by the exposure and effects of organic substances. Additionally, Cellular Energy Allocation (CEA) was determined to indicate if there are any energetic disturbances in the fish. Acetylcholinesterase concentrations decreased directly downstream of the irrigation scheme while the catalase and protein carbonyl concentrations increased. This was indicative of oxidative stress caused by reactive oxygen species due to possible pesticide exposure. However, CEA did show an increase which was attributed to increased food availability as a result of the algal growths present at the site due to nutrient loading. The site situated further downstream of the irrigation scheme did not indicate any effects or exposure to stressors that could have originated from the irrigation scheme. The active biomonitoring approach provided valuable information of the effects of pesticides and nutrient input from the irrigation scheme on the receiving aquatic ecosystem.

**RP173 Chronic Fish Toxicity of Light-Dependent Peroxidizing Herbicides in Presence of Ultra-Violet Light** A. Blankinship, T. Steeger, K. Sappington, C. Aubee, M. Grable, D. Reed-Judkins, U.S. Environmental Protection Agency. The light-dependent peroxidizing herbicides (LDPHs) consist of a group of chemicals that are used for weed control. These chemicals act by inhibiting the enzyme protoporphyrinogen oxidase (protox).

Protox exists in both plants and animals and has been shown to be sensitive to LDPHs. When aquatic organisms are exposed to a LDPH in natural settings they may be exposed simultaneously to various levels of ultra-violet light, therefore concerns have been raised that standard laboratory tests may underestimate the toxicity of LDPH in the field. To address these concerns, the EPA is currently working with a pesticide registrant task force to evaluate the chronic toxicity of LDPHs on fathead minnow (*Pimephales promelas*) fish under enhanced ultra-violet lighting conditions. Three surrogate LDPH chemicals, chosen based on persistence and chronic effects to fish, will be used for testing and the results of these studies will be used to determine a toxicity adjustment factor for fish chronic toxicity studies. Once derived, this toxicity adjustment factor will be applied to the results of a standard early life stage (ELS) (OCSPP 850.1400) and full life cycle (LC) toxicity (OCSPP 850.1500) study for both freshwater and estuarine/marine fish for all LDPH chemicals if actual toxicity data under enhanced ultra-violet lighting conditions do not exist. Preliminary results of three tests under enhanced ultra-violet lighting suggest that the potential magnitude of the toxic response is variable when compared to the results observed under standard laboratory lighting.

**RP174 Derivation of Aquatic Life Benchmarks and Quality Assurance** X. Deng, California EPA / Pesticide Regulation; K.S. Goh, California EPA / Department of Pesticide Regulation. Aquatic life benchmarks, developed by US EPA's Office of Pesticide Program (OPP), are toxicological reference values below which pesticides are not expected to harm aquatic life. These aquatic life benchmarks are extracted from the most recent publically available OPP risk assessment for the pesticide, and are based on the most sensitive acute and chronic toxicity data for each taxa. Benchmark values for acute and chronic exposures are available for freshwater fish, invertebrates, vascular and non-vascular plants. The poster demonstrates the processes in extracting the aquatic life benchmark values and multi-layer quality assurance that are involved in federal and state agencies including California Department of Pesticide Regulation. OPP launched a benchmark online database with values for 70 chemicals. Currently, the benchmark values are posted for some 375 chemicals. OPP and state agencies use the aquatic life benchmarks to make registration decisions for large number of pesticides. Attendance depending on the State approval. Final decision will be made in two weeks.

**RP175 Derivation of Sediment Quality Criteria for Pesticides** T.L. Fojut, University of California-Davis / Environmental Toxicology; M.E. Vasquez, California Water Quality Control Board – Central Valley Region / Environmental Toxicology. Pyrethroid insecticides have been detected in sediments in both urban and agricultural areas. The current (2010) Clean Water Act Section 303(d) list of impaired waterbodies for the Central Valley of California includes 13 waterbody segments with pyrethroid levels in sediments that exceed narrative water quality standards. Pyrethroids are hydrophobic organic compounds that tend to partition to sediment, and they are acutely toxic to aquatic invertebrates at low concentrations. In order to develop the numeric endpoints for total maximum daily loads required by the Clean Water Act and assess sediment concentration data for sediment pollutants, numeric criteria are desired. The U.S. Environmental Protection Agency does not have a widely used and accepted protocol for deriving numeric sediment quality criteria that is analogous to the 1985 guideline for deriving water quality criteria. Thus, the aim of this project is to develop a robust scientific methodology for deriving sediment quality criteria for the protection of aquatic life. The first phase of this project was to review methodologies for deriving sediment quality criteria from around the world, and critique them on the basis of the ability to derive chemical-specific numeric criteria. The three main approaches examined in this review were empirical, mechanistic, and spiked-sediment toxicity testing. The conclusions of this review will be presented, including techniques for incorporating bioavailability, addressing chemical mixtures, and including bioaccumulation. Based on this review, a method for deriving sediment quality criteria for pesticides will be developed in the second phase of this project, which will then be applied to generate criteria for pesticides of concern in the Central Valley of California. These criteria may be used as numeric endpoints of total maximum daily loads and for assessing data for pyrethroids and other pesticides detected in sediments.



**RP176 Development of a Spatial Dataset Representing Land Use to Support National-Level Listed Species Analysis for EPA's Registration Review of Pesticides** P. Havens, Dow AgroSciences, LLC.; M.F. Winchell, C. Hofmann, Stone Environmental, Inc.; C.M. Holmes, J.J. Amos, Waterborne Environmental, Inc.; A.R. Frank, Compliance Services International / Principal Consultant. As part of pesticide Registration Review, the U.S. Environmental Protection Agency (U.S. EPA) has recently requested registrants of some pesticide active ingredients to provide information on the distance between locations of species listed by the Endangered Species Act (ESA) and potential pesticide use sites. This proximity analysis should be based on "best available" land use and species habitat data. However, there are various data sources, methodologies and assumptions that can be combined to form a "best available" land use data set. The goal of producing such data is to provide a reasonable balance between Type I (false positive) and Type II (false negative) errors associated with the analysis. Consequently, a registrant working group explored various approaches to developing a spatial dataset representing historical land use (certain crop groups and other types of land cover and use) on a national scale. This poster examines advantages, disadvantages, and challenges of the approaches considered, assesses their impact on Type I and Type II errors, and shows how land use data can be used to generate proximity information relating potential pesticide use areas to locations of ESA-listed species that have sub-county habitat location data.

**RP177 Does measuring cell number inhibition improve an existing high throughput algal bioassay?** R. Gauch, Swiss Centre for Applied Ecotoxicology EAWAG – EPFL / EPFL; M. Junghans, Swiss Centre for Applied Ecotoxicology EAWAG – EPFL; L. Sigg, Eawag, Swiss Federal Institute of Aquatic Science and Technology; L. Werner, Swiss Centre for Applied Ecotoxicology EAWAG – EPFL. Herbicides applied in agriculture or weed control are regularly detected in samples of surface waters. Bioassays with algae can be used to assess the effects of these herbicides on non-target aquatic organisms. For routine monitoring, such bioassays should be able to screen large amounts of low volume samples and give reproducible and accurate results within a short time period. For this purpose Escher and Rutishauser (2008) developed a 24h-biotest using 96-well-plates for simultaneously assessing the photosynthesis inhibition and effects on growth in green algae measured as optical density (OD). For herbicides inhibiting the cell division of algae, such as chloroacetanilide herbicides, this test might have a blind spot since OD mainly detects effects on cell volume growth and is less sensitive for effects on cell division. To analyze the quantitative importance of this blind spot the above mentioned biotest was expanded to the additional endpoint cell number after 48h. Two substances were selected as model compounds for this study: the phenylurea herbicide diuron, which is a known photosynthesis inhibitor and is already used as a model substance in the 24h-biotest, and the chloroacetanilide herbicide metazachlor. Comparing the EC50 values of the different endpoints photosynthesis-, growth- and cell number inhibition identifies photosynthesis to be clearly the most sensitive endpoint for diuron, followed by cell number and growth. For metazachlor, cell number is most sensitive, followed by growth and photosynthesis. The determination of the EC50 for the latter two proved to be delicate due to the multi-phased dose-response-relationships observed. Predicting mixture effects with diuron and metazachlor showed that at a ratio of 1:1.66 or lower these multi-phased curves start to affect the test sensitivity. At higher ratios cell growth is already inhibited such by diuron that the effect on cell division is less sensitive than the effect on photosynthesis. Analysis of environmental samples from two rivers indicate that in the majority of cases this ratio is not achieved. Thus, the combined algae test seems to be an appropriate instrument to assess the toxicity of most environmental samples.

**RP178 Effects of 7 pesticide exposures on endocrine and biomarkers in muddy loach (*Misgurnus anguillicaudatus*)** S. Han, J. Kim, D. Yeom, Korea Institute of Toxicology / Gyeongnam Department of Environmental Toxicology and Chemistry. Pesticides have contributed significantly to improving quality of life and safeguarding the environment. However, potential toxicities of pesticides like persistent organic pollutant (POPs) to non-target species have been reported, when they were inadvertently discharged into ecosystem. In this study, 21day exposure to 7 pesticides, alachlor, benomyl, carbaryl, endosulfan, methomyl, metribuzin, 2,4-D, were performed using *Misgurnus anguillicaudatus*, which inhabit widely rice paddy in Korea, under continuous flow-through system. Following the range-finding test, test concentrations for 21d exposure were determined below the lethal level as much as possible, which is recommended by OECD test guideline for 21-day fish

assay. We also observed various biomarkers such as condition factor (CF), gonado-somatic index (GSI), liver-somatic index (LSI), vitellogenin (VTG), DNA damage using comet assay, acetylcholinesterase (AChE), and ethoxycresorufin-O-deethylase (EROD) at middle and end of the test for identifying the effect of endocrine disruption and biomarkers. VTG in blood plasma was significantly greater in male fish exposed to 0.002 mg/L endosulfan, in female and male fish exposed to 0.4 and 2 mg/L methomyl, in male fish exposed to 5 mg/L carbaryl, and in female fish exposed to 10 mg/L metribuzin for 21d than in control ( $p < 0.05$ ). While CF, GSI, and LSI weren't affected by exposure to test pesticides, DNA were damaged in fish exposed all test pesticides ( $p < 0.05$ ). AChE and EROD activity in *M. anguillicaudatus* were also influenced by exposure of carbaryl, endosulfan, methomyl, and alachlor, endosulfan, 2,4-D, respectively ( $p < 0.05$ ). In conclusion, biomarkers used in this study are helpful to evaluate influence on endocrine disruption as well as biochemical biomarkers, and this results warrant further studies on pesticides supposed potential endocrine disruptor, for example methomyl and endosulfan.

**RP179 Effects of a juvenile hormone analog, pyriproxyfen, on *Daphnia magna* in pulse exposure tests and in a two-generation test** H. Watanabe, National Institute for Environmental Studies / Center for Environmental Risk Research; R. Abe, The University of Tokyo / Graduate School of Frontier Science; S. Oda, National Institute for Environmental Studies / Center for Environmental Risk Research; N. Tatarazako, National Institute for Environmental Studies / Center for Environmental Risk Research, National Institute for Environmental Studies / Endocrine Disrupter Research Laboratory. Pyriproxyfen, widely used as a pesticide, is one of the juvenile hormone analogs (JHAs) which induce male offspring in daphnids. Previous researches revealed that male induction by JHAs occurs in a short sensitive period when oocytes mature in ovarian. To evaluate how this sensitive period of JHAs effects on the reproduction of *Daphnia magna*, we conducted 21-d reproduction test of pyriproxyfen with two exposure regimes; continuous and pulse exposure. In the continuous exposure test, *D. magna* were exposed to 50 ng/L pyriproxyfen during 21 days. Pulse-exposure tests included four single exposures and one multiple pulse exposure, in which average concentration of pyriproxyfen was designed as same as continuous exposure test. In the single pulse exposure, we exposed 525 ng/L pyriproxyfen during two days in four different growth stages; between Day0-1, Day5-6, Day10-11, and DAY15-16. In the multiple pulse exposure, we exposed 131.25 ng/L pyriproxyfen during two days four times (between Day0-2, Day5-6, Day10-11, and DAY15-16). In the results, the multiple exposures produced male offspring in the highest ratio (40%); however, the continuous exposure and single pulse exposure in the juvenile stage (Day 0-1 and Day 5-6) almost never induced male offspring (0.7%, 0%, and 4.5%, respectively). It indicated that frequent exposure in the sensitive period was an important factor in male induction. Moreover, to evaluate transgenerational effect of pyriproxyfen on reproduction, we conducted two generation reproduction test. In this test, the first generation of *D. magna* was pre-exposed to different concentrations of test chemicals for 16 days. The neonates from each test concentration continued to be exposed for additional 21 days at the same concentration respectively and designated as exposed groups. In addition, neonates from the control groups of first generation were also exposed to different test concentrations and considered as un-exposed group. Transgenerational effects of chemicals on reproduction were evaluated by comparing exposed and un-exposed group. In the results, NOEC of male production in exposed group was one concentration lower than that of un-exposed group and EC50 in exposed group was 124 ng/L which was lower than that in un-exposed group (170 ng/L). It indicated that the first generation exposure increased the induction of male offspring in the second generation. These results will provide useful data for risk assessment of JHAs in population level.

**RP180 Effects of pesticides to critical zooplankton species of the San Francisco Estuary** S.A. Lesmeister, University of California at Davis / Ecology, Veterinary Medicine: Anatomy, Physiology and Cellular Biology, University of California, Davis; S. Teh, University of California- Davis. The calanoid copepods, *Eurytemora affinis* and *Pseudodiaptomus forbesi*, are a critical link between primary producers and fish in the San Francisco Estuary (SFE). Since these meso-zooplankton play an important role as food sources to larval fish and pelagic organisms, factors affecting their changes in abundance in recent years warrants an investigation. Insecticide exposure from nearby agricultural and urban run-off may be one of several factors acting to lower pelagic productivity in the estuary. The goal of this study is to

estimate mean lethal concentrations (96-hr-LC50s) of five pesticides (Bifenthrin, Permethrin, Lambda-cyhalothrin, Chlorpyrifos and Fipronil) and to assess if ambient field water samples from critical spawning and nursery habitat are affecting the survival of *E. affinis* and *P. forbesi*. The 96-hr-LC 50 values of five pesticides investigated are near environmentally relevant concentrations. Of the five pesticides studied the most toxic to least toxic are Bifenthrin, Lambda-cyhalothrin, Permethrin, Fipronil with 96-hour LC 50 values ranging from 10 to 1000 ng/L. Chemical analyses of these pesticides are currently under investigation. Ambient water testing is a work in progress, where pilot studies indicate acute toxicity of ambient water of critical habitats to copepods. Findings from this study may lead to changes in types of pesticides used and timing of pesticide applications in the SFE. Changes in pesticide regulation may lead to a more sustainable ecosystem by decreasing the population level effects of pesticides on zooplankton, resulting in an increase of food supply to higher trophic level organisms.

**RP181 Effects of repeated exposure of a pesticide to populations of *Daphnia magna*** L. Dolciotti, UFZ Leipzig / System Ecotoxicology; M. Liess, UFZ Helmholtz Centre for Environmental Research. In the evaluation of the toxicity of substances on aquatic non target animals, test organisms are exposed either to a short contamination of 24 or 48 hours or to a chronic one in which the test system is renewed every day to maintain the medium at a constant concentration. However, standard toxicity tests do not represent entirely the way aquatic organisms are exposed to contaminants in situ. In the field we rather observe sequential pulses of contaminants due to drift or run off. Large amounts of pesticide are applied to agriculture area each year with much of it washed into adjacent water bodies. Carry-over toxicity has been observed in those organisms that survived an exposure to a toxicant but carry some damage resulting in reduced fitness. Due to a second event of exposure, stronger effects are possible when the organisms have not fully recovered (Ashauer et al. 2007). We tested this effect on populations of *Daphnia magna* in laboratory conditions. We repeatedly exposed populations of *Daphnia magna* at different intervals to a pulse of a pesticide. We reared the populations into glass vessels according to Foit et al (Foit et al. 2012) and we divided them into 3 treatments: a control, a treatment contaminated every 7 days, a treatment contaminated every 14 and a last treatment contaminated every 28 days. The contamination consisted of a pulse exposure of 24 hrs with the pesticide pirimicarb. We tested two different levels of pesticide: 14 and 24 µg/L. The experiment lasted 56 days from the first day of application. The populations of *D. magna* were monitored twice per week with an image analysis system developed by our department, and endpoints like abundance, biomass and size class were collected. The populations exposed every 7 days, showed not increased mortality for both the concentration tested. After a second pulse of the contaminant we expected the populations to be more sensitive at the lowest interval. However, for the interval of 7 days we observed that less individuals died compared to those contaminated after 28 days. Moreover the highest mortality recorded was that one of the 14 days interval. Additional applications at 7 days interval and at the highest contamination drove some populations to extinction. We conclude that for *D. magna* there were no carry-over effects for the pesticide pirimicarb and that the mortality and recovery of the population was due to differences in the size structure of the population during contamination.

**RP182 Effects of vitamin C on the developmental toxicity of chlorpyrifos in zebrafish** F. Rubio-Escalante, E. Norena-Barroso, Universidad Nacional Autonoma de Mexico / Unidad de Química Sisal; D. Schlenk, University of California-Riverside / Department of Environmental Sciences; G. Rodriguez-Fuentes, Universidad Nacional Autonoma de Mexico / Unidad de Química Sisal. Acetylcholinesterase (AChE) is inhibited by numerous pollutants including organophosphate pesticides (OPs). OPs cause the irreversible inhibition of AChE in the central and peripheral nervous systems resulting in the accumulation of acetylcholine and excessive activation of muscarinic and nicotinic receptors, which may lead to death. Thus, dogma indicates that the target of OP pesticides is AChE, but many authors postulate that these compounds also disturb cellular redox processes, and change the activities of antioxidant enzymes. Vitamin C is a free radical scavenger that counters the persistent effect of reactive oxygen species. Interestingly, it has also been reported that oxidative stress also plays a role in the regulation and activity of AChE. In this study we evaluated the effect of vitamin C during co-exposure with chlorpyrifos. Zebrafish embryos were co-exposed with 50, 100, 200 and 400 mg/L of chlorpyrifos and 10 mg/L

of Vitamin C. Embryos were evaluated at 48 hours post fertilization for AChE activities and developmental toxicity. Endpoints used for assessing developmental toxicity included embryo survival, hatching rate, heartbeat (visually determined as the average for five embryos, counted for 15 s under a temperature-controlled stage), pericardial sac edema and yolk sac edema.

**RP183 Fate and effects of an insecticide formulation** C. de Perre, W. Hanson, D. Banner, B. Hanson, Y. Ding, M. Lydy, Southern Illinois University. The fate and toxicity of an insecticide formulation have been investigated over a two-year period on a corn field in Illinois. The active ingredients of this formulation are the pyrethroid insecticide cyfluthrin and organophosphate insecticide phostebupirim. Cyfluthrin, a sodium-potassium channel blocker neurotoxin, is considered highly toxic to marine and freshwater organisms. Phostebupirim, an acetylcholinesterase inhibitor neurotoxin, is considered highly toxic to fish and aquatic invertebrates. While the toxicity is relatively well known for each compound, the possible interaction of these two insecticides and their combined toxicity has not yet been documented for non-target species. Acute laboratory toxicity tests have been performed to assess individual and mixture effects to several non-target species including the aquatic amphipod (*Hyalella azteca*), microcrustean (*Daphnia magna*), zebrafish (*Danio rerio*) and fathead minnow (*Pimephales promelas*). The median lethal concentrations (LC50, concentrations that results in 50% organism mortality) and the median effective concentrations (EC50, concentrations that results in 50% affected organisms) were calculated for each species. Preliminary results showed that the four species tested have very different sensitivities to these insecticides. *Danio rerio* was the least sensitive with LC50 values higher than the highest concentrations tested (4.5 µg/L for cyfluthrin and 4,500 µg/L for phostebupirim). *Pimephales promelas* was more sensitive with LC50 values of 1.4 µg/L for cyfluthrin and 3,965 µg/L for phostebupirim. *Hyalella azteca* was the most sensitive species to cyfluthrin with a LC50 below 4 ng/L and *Daphnia magna* the most sensitive to phostebupirim with a LC50 value of 100 ng/L. Interaction effects of these two insecticides in combination have also been investigated. Preliminary results showed no synergism or antagonism of these two compounds for *Hyalella azteca* and *Daphnia magna*. In order to assess the environmental risk of the use of this insecticide formulation in field conditions, LC50 and EC50 values will be compared to soil and run-off water and sediment concentrations of each insecticide measured throughout the two farming seasons.

**RP184 Gene and protein expression in *Daphnia magna* exposed to glyphosate and methidathion for biomarkers screening** T. Le, Chonbuk National University / Dept. of Bioprocessing Engineering; N. Hong, Chonbuk National University; Y. Kim, Chungbuk National University; J. Min, Chonbuk National University. In this study, proteomic and transcriptomic analysis in a water flea, *Daphnia magna*, were performed for screening biomarkers to detect toxicity of glyphosate and methidathion pesticides. The LC50 and LC75 concentrations of glyphosate and methidathion, which were determined as 234 ppm, 250 ppm and 0.044 ppm, 0.054 ppm, respectively, were used to expose to the 21d daphnia organisms for 24 h prior to isolate total protein and RNA. The proteomic profile was examined with a pH range from 3 to 10 using 2DE method and then analyzed with Progenesis software to explore the differentially expressed proteins (DEPs). The results showed that there were 10 up-regulated and 12 down-regulated proteins in daphnia by the glyphosate toxicity while 14 and 25 proteins were up- and down-regulated by the methidathion toxicity. All those differentially expressed proteins were identified by MALDI-TOF. Simultaneously, the gene expression in the exposed daphnia was investigated using a cDNA microarray containing 32 DNA oligonucleotides. The combined proteomic and transcriptomic approach is potential to discover novel biomarkers as well as provide a better insight into the action mode of the glyphosate and methidathion pesticides.

**RP185 Genotoxicity of Herbicide Mixtures at Environmentally Relevant Concentrations in Texas High Plains Playa Lakes** W.H. Mimbs, N. Ghosh, West Texas A&M University / Department of Life, Earth, and Environmental Science; G.C. Barbee, West Texas A&M University / Life, Earth & Environmental Sciences. The genotoxicity of mixtures of pesticides commonly applied in the Texas High Plains have not been characterized at environmentally relevant concentrations. The freshwater crustacean *Ceriodaphnia dubia* (*C. dubia*) was used in an acute, 48-hour exposure Comet assay to assess the genotoxicity of two cotton herbicides, diuron and



metolachlor. *C. dubia* was exposed to each herbicide separately and in mixtures at environmentally relevant concentrations detected in playa lakes of the Texas High Plains. The Comet assay exhibits genetic damage as a "tail" of DNA extending from the cell body, such that it resembles a "comet." The ratio of the width to the length of the comet body/tail is used to assess the degree of genotoxicity. In *C. dubia*, the genotoxicity of the individual herbicides was negligible (ranging from 0.5-2 mg/L metolachlor and 1.75-7 mg/L diuron) compared to triethylene glycol (TEG), the negative solvent control (7 mg/L). However, the herbicide mixtures (primarily using a 3:1 environmentally relevant ratio) exhibited appreciable genotoxicity and had body/tail ratios similar to methyl methanesulfonate (MMS; 8 mg/L), the positive control. This suggests a possible synergistic effect that might not be observed if only individual chemicals are assayed, or if only chemical analysis (e.g., GC-MS) alone is relied upon to assess ecological health risks. Thus, non-target organisms inhabiting playa lakes adjacent to agricultural fields may be subject to DNA damage as a result of these, and other, herbicide mixtures. We are also currently investigating the reproductive affects (i.e., brood size and number) in *C. dubia* of these pesticides and their mixtures.

**RP186 Impacts of hypersaline acclimation on the acute toxicity and metabolism of chlorpyrifos in salmonids** L.A. Maryoung, University of California – Riverside / Environmental Science, University of California Riverside / Department of Environmental Sciences; R. Lavado, University of California Riverside / Department of Environmental Sciences, University of California Riverside / Department of Environmental Science; D. Schlenk, University of California-Riverside / Department of Environmental Sciences. Acclimation to hypersaline environments has been shown in previous studies to enhance the acute toxicity of thioether organophosphate pesticides to different fish species. The present study focuses on acute toxicity of the organophosphate chlorpyrifos after hypersaline acclimation. Rainbow trout were exposed concurrently to two salinity treatments (< 0.5 ppt and 16ppt) and five chlorpyrifos treatments (20, 40, 80, 100, and 150 µg/L). The median lethal time was significantly different between the salinity treatments at the three highest chlorpyrifos concentrations, with time to death by chlorpyrifos being more rapid in freshwater than hypersalinity. Differences in inhibition of acetylcholinesterase by the oxon metabolite between freshwater and hypersaline acclimated fish were explored as a factor in time to death. The IC<sub>50</sub> was 84.5 ± 26.3 and 72.01 ± 19.2 nM for freshwater and hypersaline acclimated fish respectively, showing no difference. Hypersaline conditions enhanced the expression of cytochrome P450 3A in previous studies. To determine whether CYP3A may detoxify chlorpyrifos through cleavage of the phosphoester bond, rather than desulfuration to oxon metabolites, the ratio of the detoxified metabolite to the activated metabolite was measured in freshwater (1.47 ± .40) and hypersaline (1.43 ± .68) acclimated fish. Regression analysis with CYP3A expression and the cleavage detoxified product failed to show a significant relationship. These results indicate hepatic biotransformation may not play a role in the difference in time to death in saltwater acclimated fish and that other factors may be responsible for the enhanced toxicity of chlorpyrifos in freshwater acclimated fish (NIEHS P30ES07033).

**RP187 Impacts of hypersaline acclimation on the acute toxicity, estrogenicity, and the biotransformation of bifenthrin in *Oncorhynchus mykiss*** N. Riar, University of California Riverside / Department of Environmental Sciences; K.L. Forsgren, University of California, Riverside / Department of Environmental Sciences, University of California / Dept of Environmental Sciences; D. Schlenk, University of California-Riverside / Department of Environmental Sciences. Bifenthrin, a pyrethroid pesticide, is acutely toxic to non-target aquatic organisms. Studies have shown that pyrethroids and their metabolites increase estrogenic activity via vitellogenin (VTG) production in male fish. Due to its widespread urban use, bifenthrin is frequently measured in the San Francisco Bay Estuary and Sacramento-San Joaquin Delta (SFBD). The unique life history of salmonids, migrating between freshwater and seawater, makes them particularly susceptible to multiple stressors in the waterways they pass through including pesticide exposure and salinity. The current study investigated the effects of environmentally relevant concentrations of bifenthrin in two populations of *Oncorhynchus mykiss*; Jess Ranch fish (JR) and Nimbus Hatchery fish (NH). Acute toxicity, vitellogenin protein (VTG), estradiol-17β (E2), and the biotransformation of bifenthrin were studied in JR and NH fish were acclimated to freshwater (FW) and hypersaline conditions (8 and 17ppt) and exposed to bifenthrin (0, 0.1, and 1.5 µg/L) for 14 days. FW acclimated

JR fish exposed to 1.5 µg/L bifenthrin experienced significantly greater mortality (47.22 ± 24.06% survival; p=0.0336) compared to low-dose bifenthrin (0.1 µg/L) JR fish and bifenthrin-treated NH fish in both FW and under hypersaline conditions. There was no significant difference in plasma VTG levels in JR fish (p=0.4332) and VTG levels were below detection in NH fish. There was no significant (p=0.3769) difference in E2 levels in bifenthrin-treated JR fish in FW or under hypersaline conditions. A significant (p=0.0049) increase in E2 was observed in FW-acclimated NH fish exposed to 1.5 µg/L bifenthrin. There was a significant (p=0.0271) decrease in bifenthrin metabolites in liver microsomes from JR fish acclimated to 17 ppt. There was no significant (p=0.8140) difference in bifenthrin metabolites in liver microsomes from NH fish in FW or acclimated to 17 ppt. Overall, this study indicates that 14-day bifenthrin exposure may not increase VTG, however previous exposure to estrogenic compounds during early development (JR fish are treated with E2 as embryos to obtain an all female population for stocking purposes) may increase salmonid susceptibility to bifenthrin in FW.

**RP188 Methodology for Evaluating Pesticides for Surface Water Protection** Y. Luo, California Department of Pesticide Regulation; X. Deng, S. Gill, K.S. Goh, California Department of Pesticide Regulation. The Surface Water Protection Program of California Department of Pesticide Regulation is developing a more consistent and transparent method for evaluating registration packages. Historically, these evaluations have been based principally on professional judgment and experience gleaned from past assessment of the conditions and mechanisms responsible for the offsite transport of pesticides to surface water and their associated toxicological impact on aquatic life. A two-stage procedure is proposed, including stage I evaluation with initial screening, and stage II evaluations with refined modeling. Initial screening is conducted solely on chemical properties (soil adsorption coefficient, water solubility, and reaction half-lives) and aquatic toxicology data of the active ingredient. For pesticides which may potentially cause surface water problems, stage II evaluation is performed for additional evaluation by predicting pesticide exposure and risk at the edge of fields based on refined modeling approach. The evaluation results are summarized as registration recommendations, i.e., [1] to support registration without conditions for pesticides which are unlikely to be a surface water quality problem, [2] to support conditional registration with requests for analytical methods for pesticides which may potentially cause surface water problems, or [3] not to support registration for pesticides which pose unacceptable potential surface water impacts. Notes to the meeting coordinator: This abstract and presentation in SETAC are pending for State approval. The final decision will be made in a couple of weeks.

**RP189 Mitigating with macrophytes: A comparative look at insecticide mitigation by four species of submersed aquatic plants** W.R. Brogan, University of Pittsburgh / Biological Sciences; R. Relyea, University of Pittsburgh / Department of Biological Sciences. Understanding the influence of aquatic macrophytes on contaminant effects in aquatic ecosystems is an important contemporary goal in ecotoxicology. Previous studies have demonstrated the ability of some species of submersed macrophytes to remove some contaminants, including many insecticides, from the water column. However, the degree to which macrophytes actually mitigate insecticide toxicity to sensitive aquatic animals, and the generalizability of insecticide mitigation ability across submersed plant species are poorly understood. In this study, we compared the toxicity of a low (3.3 µg/L) and high (23.7 µg/L) concentration of the organophosphate insecticide malathion (as well as insecticide-free controls) to the keystone aquatic herbivore *Daphnia magna* in the absence of macrophytes, in the presence of monocultures of the submersed macrophytes *Elodea canadensis*, *Myriophyllum spicatum*, *Ceratophyllum demersum*, and *Vallisneria spiralis*, as well as in the presence of two types of inert substrates (plastic aquarium plants and rope) to control for the surface area provided by the live macrophytes. In addition to quantifying each macrophyte treatment's effects on malathion's toxicity, we also examined how quickly the toxicity of each malathion concentration decreased in each macrophyte treatment by exposing *Daphnia* to samples of water extracted from each microcosm at 2, 8, and 48 h after dosing. We discovered that while both low- and high-malathion concentrations decimated *Daphnia* in the no-macrophyte, plastic plant, and rope treatments, all living macrophyte species exhibited equal abilities to strongly mitigate malathion's lethality. Further, water treated with low- and high-malathion concentrations remained highly toxic to *Daphnia* for 48+ h in the no-macrophyte,



plastic plant, and rope treatments, whereas the living macrophytes all reduced malathion's toxicity to control levels within 8 h. These results demonstrate not only the strong mitigating effects that living macrophytes have on insecticide toxicity, but also that this mitigating influence is not merely a result of the surface area provided by plant shoots. Finally, our study provides evidence that insecticide mitigation is generalizable across numerous macrophyte species.

**RP190 Quantification of imidacloprid residues in green sturgeon following application of the insecticide in Willapa Bay, Washington** J.A. Frew, Washington Cooperative Fish and Wildlife Research Unit, University of Washington / School of Aquatic and Fishery Sciences, University of Washington / School of Aquatic and Fishery Sciences, University of Washington, Washington Cooperative Fish and Wildlife Research Unit / School of Aquatic and Fishery Sciences; C. Grue, Washington Cooperative Fish and Wildlife Research Unit, University of Washington / School of Aquatic and Fishery Sciences, USGS, Washington Cooperative Fish and Wildlife Research Unit / University of Washington. The southern DPS of green sturgeon was listed as "threatened" under the Endangered Species Act in 2006, followed by the establishment of critical habitat in 2009 ranging from central California north along the coast to the U.S. border with Canada. Use of the insecticide imidacloprid (IMI) has been proposed for the control of burrowing shrimp that impact commercial oyster beds in Willapa Bay and Grays Harbor, Washington; areas where adult green sturgeon aggregate during the summer and autumn. Federal registration and State permitting approval of IMI for burrowing shrimp control may be threatened if it is determined that its application adversely affects these fish. Observations following test applications of IMI on intertidal beds indicate that green sturgeon feed opportunistically on contaminated shrimp. These fish may be exposed to IMI while digging for and consuming shrimp following application of the insecticide. Environmental monitoring was conducted following test applications of IMI on intertidal beds in Willapa Bay in July 2010. Blood samples were collected and plasma isolated from 32 green sturgeon caught and released at sites proximal to IMI test beds at approximately 6 and 30 h following application. IMI residues were detected in five samples following an initial ELISA screening. LC-MS/MS detection will be used for definitive quantification of these samples. Additional monitoring is planned in Willapa Bay in July 2012 following test applications of IMI. In addition, surrogate juvenile seawater-adapted white sturgeon were exposed to concentrations of IMI for durations simulating expected worst-case scenarios in the field over three successive feeding intervals (6, 18 and 30 h) post application. No overt effects were observed. Average IMI residues were 22.3, 32.7, and 32.7  $\mu\text{g kg}^{-1}$  ( $n = 4, 6, \text{ and } 4$ , respectively). These values represent an expected ceiling for IMI concentrations in green sturgeon measured during environmental monitoring. Results from these environmental monitoring studies will be incorporated into an evaluation of the risk to green sturgeon from IMI through estimation of exposure, uptake, distribution, elimination and potential toxic effects of the compound.

**RP191 Risk Assessment for Adult Butterflies Exposed to a Mosquito Control Pesticide on the National Key Deer Wildlife Refuge, Big Pine Key, Florida** T. Bargar, U.S. Fish & Wildlife Service / U.S. Geological Survey / Southeast Ecological Science Center. A series of laboratory and field studies have been conducted to assess the risk to imperiled butterflies (Florida leafwing [*Anaea troglodyta floridae*] and Barram's hairstreak [*Strymon acis bartrami*]) on the National Key Deer Wildlife Refuge (Refuge) from aerial organophosphate pesticide (naled) applications to control adult mosquitos on the Refuge. Laboratory toxicity studies on four butterfly species determined that (1) the minimum cholinesterase (ChE) inhibition levels associated with significant mortality ranged from 26 to 67% of reference, and (2) the ChE activity level significantly less than reference ranged from 8.4 to 12.3  $\mu\text{M/min}^*\text{g}$  depending on the species. Further laboratory studies demonstrated that while butterflies are responsive (cholinesterase activity and mortality) to a dose on the wings, it is inversely related to the distance between the body and the dose location on the wings. Naled dosed to the thorax and to the proximal, medial, or distal portions of the forewings of adult butterflies (Julia [*Dryas julia*]) resulted in LD50 values of 7.6, 5.4, 35.9, and 82.2  $\mu\text{g/g}$ , respectively. Using the variable response data for wing exposure, a modified effect level was derived to account for exposure over the entire butterfly body (wings and central body), which is likely for butterflies at locations treated by ultra-low volume sprays such as the Refuge. In addition, an exposure metric was derived to equate the units for pesticide residue

data based on surface area (i.e.,  $\mu\text{g/m}^2$ ) collected during field studies to the units typical for effect levels ( $\mu\text{g/g}$ ) in order to assess risk. Comparison of the pesticide residue data from the field studies to sensitivity distributions (mortality and ChE activity) corroborated effects data measured for butterflies during the same field studies; that there is a high level of risk for the imperiled butterflies on the Refuge due to the aerial naled applications.

**RP192 Short-term effects of propiconazole on hypothalamic-pituitary-gonadal function in the fathead minnows (*Pimephales promelas*)** S.Y. Skolness, University of Minnesota / Department of Biochemistry and Molecular Biology; K. Jensen, U.S. EPA / National Health and Environmental Effects Research Laboratory; C. LaLone, U.S. EPA; G.T. Ankley, US EPA / Office of Research and Development, National Health and Environmental Effects Research Laboratory, Mid-Continent Ecology Division, U.S. EPA / National Health and Environmental Effects Research Laboratory; D.L. Villeneuve, U.S. EPA / National Health and Environmental Effects Research Laboratory, U.S. EPA; E. Makynen, US EPA / Mid-Continent Ecology Division; E. Durhan, US EPA / Mid-Continent Ecology Division, US-EPA / Research Chemist; M. Kahl, U.S. EPA / National Health and Environmental Effects Research Laboratory. Propiconazole is an ergosterol inhibitor commonly used in agriculture and has been detected in aquatic environments. Ergosterol inhibitors decrease fungal growth through effects on 14 $\alpha$ -demethylase, a cytochrome P450 (CYP), isoform important for ergosterol biosynthesis. In higher organisms, other CYPs are targets for ergosterol inhibiting compounds, such as CYPs involved in sex steroid synthesis or those responsible for Phase I xenobiotic metabolism. To determine the short-term effects of propiconazole on endpoints related to function of the hypothalamic-pituitary-gonadal axis, adult female fathead minnows (*Pimephales promelas*) were exposed to 0 or 1000  $\mu\text{g/L}$  of the fungicide via the water. The fish were treated for up to 24 hours, with sampling at 6, 12, and 24 hours when plasma, liver and ovary were collected. Ovarian *ex vivo* production of testosterone and 17 $\beta$ -estradiol (E2) and plasma E2 were determined by radioimmunoassay. Additionally, quantitative real-time PCR was used to examine expression of a number of genes in ovary and liver. Propiconazole rapidly reduced circulating E2 concentrations and *ex vivo* E2 production, but had no effect on the ovarian expression of genes coding for proteins related to steroidogenesis, including steroidogenic acute regulatory protein and CYP19 aromatase. Hepatic expression of *cyp11a1*, which is involved in metabolism of xenobiotics, was significantly up-regulated at all time-points. This study will aid in understanding the short-term responses of the liver-ovary axis to endocrine disrupting chemicals.

**RP193 Testicular oocyte presence and severity differs across amphibian species, unrelated to atrazine concentration** J. Olker, University of Minnesota / Natural Resources Research Institute; R. Johnson, U.S. Environmental Protection Agency / ORD/NHEERL/MED; P. Schoff, L. Johnson, University of Minnesota Duluth / Natural Resources Research Institute. The presence of egg cells in testicular tissue (testicular oocytes, TOs) has been linked to exposure to endocrine disrupting chemicals (EDC), and TO presence and abundance has been used as a biomarker for EDC exposure in amphibians and fish. Our main objective was to assess the effects of atrazine exposure on testicular oocyte presence, abundance, and severity in reared and wild metamorphic frogs from two native species, *Rana pipiens* (Northern leopard frog) and *Rana sylvatica* (wood frog). Tadpoles of both species were chronically exposed to environmentally relevant atrazine concentrations (0.1, 20, 200  $\mu\text{g/L}$ ) through metamorphosis in outdoor mesocosms. In addition, wild metamorphic *R. pipiens* were collected from ten wetlands in the Prairie Pothole Region (PPR) with atrazine concentrations ranging from below detection limit (0.011  $\mu\text{g/L}$ ) to 0.855  $\mu\text{g/L}$ . TOs were identified, counted, and severity assessed against a validation set in longitudinal 50  $\mu\text{m}$  step-sections covering the entirety of both gonads. TOs were found in both species, with different frequencies of occurrence (>50% in *R. pipiens* and < 5% in *R. sylvatica*). No relationship was found between atrazine concentrations and TO presence, abundance, or severity in either experimental or field-collected *R. pipiens*. Two additional objectives developed from the unexpected species' differences: 1) Assess the influence of developmental stage on TO prevalence, and 2) Evaluate TO detection probability within specimens and wetlands based on current protocol. To assess whether low prevalence of TOs in *R. sylvatica* was related to gonadal development, we evaluated specimens from one northern Minnesota wetland collected from pre-metamorphosis to two months post-metamorphosis. Gonads from these specimens were clearly differentiated at metamorphosis

and contained no TOs before or after metamorphosis. Wild *R. pipiens* were used to evaluate TO distribution and detection probability. TOs were found in *R. pipiens* at all ten PPR wetlands and, when present, TOs were evenly distributed throughout the middle portion of both gonads. Collecting fewer specimens per site or fewer sections per specimen resulted in comparable TO prevalence, abundance, and severity, suggesting two effective time- and cost-saving methods for evaluating amphibian TOs. Given the species differences in the prevalence of TOs, their as indicators of endocrine disruption or to evaluate environmental condition should be regarded with caution.

**RP194 The Emerging Process of Pesticide National-Level Endangered Species Analysis in Support of FIFRA Registration Review** B.D. McGaughey, Compliance Services International; T. Hall, Bayer CropScience / Ecotoxicology; D. Campbell, Syngenta Crop Protection. Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) risk assessments required in Registration Review, to evaluate the potential risk of pesticides to species listed under the Endangered Species Act (ESA), are a tremendous challenge, considering the Registration Review schedule, the broad spatial scale of the action, and the available resources of the Environmental Protection Agency (EPA) Office of Pesticide Programs (EPA-OPP). The ESA and the agencies charged with administering it are foundationally centered on the evaluation of a site-specific agency action involving a limited number of listed species, compounding the challenge of ESA consultation when it is requested by EPA-OPP for broad scale use of a pesticide where multiple sites and species are involved. This poster examines how the national process is presently operating in the early stages of Registration Review. It also considers the questions put before the National Academy of Sciences by the Services and EPA with respect to the scientific methodology that should be used in national level ESA risk assessments, which result in species-specific effects determinations. Finally, the poster considers how national level assessment might progress to highly specific local implementation by exploring the application of a tiered process that moves from a national screening level assessment, suitable to address Registration Review needs, to a site specific implementation process, suitable to address the needs of the local user community and protection of the endangered species.

**RP195 Antihepatotoxic activity of *Sapium ellipticum* stem bark against carbon tetrachloride (CCl<sub>4</sub>) induced liver toxicity on experimental rats** A. Njouendou, University of Buea/Cameroon / Biochemistry and Molecular Biology; P. Nkeng-Efouet-Alango, University of Dschang/Cameroon / Chemistry; J. Assob Nguenda, University of Buea/Cameroon / Biomedical Sciences; B. Shrishailappa, Sree Siddaganga College of Pharmacy / Pharmaceutical Chemistry; S. Wanji, University of Buea/Cameroon. Background: Carbon tetrachloride (CCl<sub>4</sub>) belongs to a family of common organo-halogen toxins found in the environment and is toxic to the liver of animals. Its toxicity is mediated by the ·CCl<sub>3</sub> free radical, release after its biotransformation by liver Cytochrome P-450 oxidase enzyme complex. This radical induces the degradation of macromolecules such as proteins, lipids and nucleic acids which leads to the lesion of hepatocytes. It has been shown that antioxidants can scavenge free radical and protect the cell against damage. *Sapium ellipticum* bark is a part of plant used in phyto-medicine for the treatment of liver disease. Its *in vitro* antioxidant property has been proved. Objective: To evaluate the protective effect of *Sapium ellipticum* stem bark extract against carbon tetrachloride (CCl<sub>4</sub>) induced liver toxicity. Materials and methods: The crude extract of *Sapium ellipticum* was suggested to acute toxicity on female mice according to the OCDE guidelines. Hepatotoxicity was induced in female Wistar rats by intraperitoneal injection of single dose (CCl<sub>4</sub> in liquid paraffin 1:1 v/v, 1.25 ml/kg) after 7 days of treatment. Methanol extract of *Sapium ellipticum* stem bark dissolved in 1% gum acacia was administered to the animals at dose 100 mg and 200 mg/kg, p.o. daily for 12 days. The anti-hepatotoxic effect of these extracts was evaluated by the assay of serum biochemical parameters of liver function (serum albumin, total bilirubin, serum protein, alanine aminotransaminase, aspartate aminotransaminase, and alkaline phosphatase), tissues antioxidant parameters and histopathological studies of the liver. Results: The toxicity of CCl<sub>4</sub> was observed in toxic group by increase of liver enzymes in serum, decrease of serum proteins and liver antioxidant markers. In methanol extract-treated animals, the toxic effect of CCl<sub>4</sub> was controlled significantly by restoration of the levels of serum bilirubin, protein, enzymes and antioxidant parameters as compared to the normal and the standard drug Silymarin treated groups. Histology of the liver sections of the animals treated with the extracts showed the presence of normal hepatic cords, absence of necrosis and fatty infiltration, which

further evidenced the hepatoprotective activity. Conclusion: Methanol extract of the stem bark of *Sapium ellipticum* possesses antihepatotoxic properties against CCl<sub>4</sub> induced liver damage.

**RP196 Effects of 2, 4-Dichlorophenoxyacetic Acid Herbicides on the Reproduction and Development of Fathead Minnows** Z.A. DeQuattro, University of Wisconsin / Zoology; W. Karasov, University of Wisconsin-Madison / Forest and Wildlife Ecology. The aim of the present study is to assess the effects of 2,4-Dichlorophenoxyacetic acid herbicide formulations on fathead minnow reproduction and early development using a variant of the fish short-term reproduction assay, an embryo development assay, and a larval exposure. Reproductively mature fathead minnows were exposed for 28 days to 0.00, 0.05, 0.50, and 2.00 ppm 2, 4-D aquatic herbicide in a flow-through system and various reproductive endpoints (i.e., fecundity and fertilization success) were assessed. At 28 days animals were euthanized, weighed, and secondary sexual characteristics (e.g., tubercles on males) were scored. Livers, gonads, and body tissues were collected for hepatic vitellogenin mRNA expression analysis and tissue burden analysis. Early development and hatch success were assessed by incubating fertilized eggs at the above concentrations until hatch. Exposure to the first formulation tested resulted in an ordered dose dependent decrease in fecundity with reductions in egg production as high as 26%. Exposure also caused a significant decrease in tubercle score in all treatments. Analysis of hepatic vitellogenin mRNA expression, tissue burden analysis, and larval survival are pending. These data, as well as the assessment of a second 2,4-D herbicide formulation (in progress) and future directions will be discussed.

**RP197 Investigation into Toxicity of Chemical Mixtures Involving Organophosphate Pesticides: Malathion and Diazinon Toxicity to *Daphnia Magna*** A. MacLeod, Western Washington University / Department of Environmental Sciences; W. Landis, A. Markiewicz, Western Washington University. Freshwater habitats in urban and agricultural regions are commonly found to contain chemical mixtures from several nonpoint runoff sources. The suburbanization of land use in close proximity to agricultural regions allows contaminants applied to be transported into the nearest waterway. The common over-application of pesticides by landscaping and agricultural practices causes excess toxicant to be washed away with runoff. Recent studies on mixture toxicity have concluded that single toxicant risk assessments may be underestimating toxicity of certain pesticides to aquatic organisms when mixing in aquatic environments. To manage the implicit risk it is critical to understand how individual chemical toxicity mixtures can affect the survival and behavior of non-target organisms. Organophosphate pesticides inhibit the activity of acetylcholinesterase (AChE) and can generate effects that can interfere with freshwater communities by alteration of lower trophic levels. Due to reported synergistic toxicity of organophosphate mixtures, it is critical to assess mixture toxicity. In this experiment 48-hour acute *Daphnia magna* toxicity tests were conducted using >98% analytical grade malathion and >98% analytical grade diazinon. The chemicals were tested at the following concentration ranges: malathion 0.70 – 11.20 µg/L and diazinon 0.37 – 6.00 µg/L. A mixture of malathion and diazinon also were tested to evaluate the potential additive, synergistic, or antagonistic toxicity: malathion 0.70 – 11.20 µg/L in the presence of 0.75 µg/L of diazinon. The mortality results indicated potential antagonism as determined by probit nonlinear regression.

**RP198 Monitoring Exposure of Green Sturgeon to Carbaryl Following Applications to Control Burrowing Shrimp in Washington State** A.T. Troiano, Washington Cooperative Fish and Wildlife Research Unit / School of Aquatic and Fishery Sciences, University of Washington, University of Washington / School of Aquatic and Fishery Sciences, University of Washington; C. Grue, Washington Cooperative Fish and Wildlife Research Unit, University of Washington / School of Aquatic and Fishery Sciences, USGS, Washington Cooperative Fish and Wildlife Research Unit / University of Washington; M. Hooper, USGS, Columbia Environmental Research Center / Columbia Environmental Research Center. Willapa Bay is one of the rare intertidal locations where large-scale pesticide applications occur. Since the 1960s, carbaryl has been applied to the mudflats in the Bay to control populations of burrowing shrimp (*Neotrypaea californiensis* and *Upogebia pugettensis*) that decrease oyster (*Crassostrea gigas*) productivity. Green sturgeon (*Acipenser medirostris*) are present in the Bay, and population declines (Southern DPS) have resulted in their listing under the ESA. White sturgeon (*Acipenser transmontanus*) are also found in the Bay, are closely



related to green sturgeon, but are not ESA listed. We exposed five seawater-adapted white sturgeon (mean wt = 1.1 kg) to each of six concentrations of carbaryl (0, 30, 100, 300, 1000 and 3000 ppb) for 6 h and measured brain acetylcholinesterase (AChE) activity. An additional cohort of smaller fish (mean wt = 0.5 kg) was also exposed to the high concentration for 6 h. Enzyme recovery was measured in additional cohorts (n=4 sturgeon, mean wt = 1.8 kg) exposed to 1000 ppb for 6 h with AChE activity measured at 0, 24, 48 and 72 h post exposure. Enzyme activity was reduced 15% in sturgeon exposed to 30 ppb for 6 h compared to controls, 27% (100 ppb), 32% (300 ppb), 35% (1000 ppb) and 42% (3000 ppb). Inhibition in the smaller sturgeon exposed to 3000 ppb for 6 h was 50%. In the recovery fish, AChE was inhibited 36% at 0 h post exposure, 23% at 24 h, 28% at 48 h, and 13% at 72 h. Comparison of the results of the brain AChE assays and measured water column concentrations of carbaryl post application in Willapa Bay suggest this route of exposure likely does not pose a hazard to the much larger green sturgeon in the Bay. Because the listed status of the green sturgeon prevents destructive sampling, we will also measure plasma (butyrylcholinesterase [BuChE]) activity in each of the cohorts of white sturgeon described above. Additionally, we will spike clean white and green sturgeon plasma with different concentrations of carbaryl to develop and compare sensitivity profiles for the two species. Results of the plasma studies will be reported. We hope plasma cholinesterase activity will be an effective biomarker for exposure of green sturgeon to carbaryl within Willapa Bay, and cholinesterase inhibitors throughout their range that includes Puget Sound, and the Columbia, Yakima, Willamette, Sacramento, and San Joaquin river systems.

**RP199 The Effect of Atrazine and Roundup® on *Daphnia Magna*** J. Stiles, Western Washington University / Huxley College of the Environment; C. Hixon, G. Lowery, W. Landis, Western Washington University. In a previous trial, we observed a stimulation of reproduction in the presence of the herbicides, so we're looking to assess whether there's an effect on reproduction in the F1 generation. To follow up, we are conducting a 21 day chronic exposure of atrazine and Roundup®, two prevalent herbicides in the United States, to *Daphnia magna*. Examining the consequences of herbicide interactions is important for understanding the potential synergistic effects of agricultural runoff mixtures. Five concentrations of atrazine are being tested (0.50, 1.00, 1.25, 1.50, 2.00mg/L) and the mixture treatments are being conducted with atrazine at those concentrations along with Roundup® (450 mg/L, 2% glyphosate). Effects on behavior and reproduction are being monitored several times a week with reconstitution of the treatment solutions once every week. Curve fitting will be used to examine the concentration-response relationships. From this experiment we will be able to examine the chronic effects of Roundup® and atrazine concentrations separately, as well as combined. We will present our updated results.

**RP200 Acute sensitivity of freshwater mollusks to select chemicals with various toxic modes of action** N. Wang, U.S. Geological Survey / Columbia Environmental Research Center; C.G. Ingersoll, USGS / Columbia Environmental Research Center, U.S. Geological Survey / Columbia Environmental Research Center; C.D. Ivey, U.S. Geological Survey / Columbia Environmental Research Center, US Geological Survey / Toxicology; E. Hammer, US Environmental Protection Agency; C. Bauer, US Environmental Protection Agency; T. Augspurger, US Fish and Wildlife Service; S. Raimondo, United States Environmental Protection Agency / Gulf Ecology Division, National Health and Environmental Effects Laboratory, US EPA / Gulf Ecology Division; B. Shephard, U.S. EPA / Office of Env. Assessment (OEA-095); J. Bartoszek, US Fish and Wildlife Service; C. Barnhart, Missouri State University / Professor; N. Eckert, US Fish and Wildlife Service. Nearly 70% of the 300 unionid mussel species in North America are endangered, threatened, of special concern, or already extinct. Environmental contamination has been identified as a causal or contributing factor to these declines of mussel populations. Previous studies indicate that mussels are more sensitive to some chemicals, such as copper and ammonia, than commonly tested organisms. However, freshwater mollusks are generally under-represented in toxicity databases. Studies are needed to generate a more comprehensive database to compare the sensitivity of mollusks to other invertebrates, and to evaluate the degree to which existing or proposed US Environmental Protection Agency (EPA) ambient water quality criteria (AWQC) are protective of mollusks relative to Endangered Species Act consultations between EPA and the US Fish and Wildlife Service (FWS). The objectives of this ongoing study are to evaluate sensitivity of select species of

mussels and snails, and two commonly tested crustacean species (amphipod, *Hyalella azteca*, and cladoceran, *Ceriodaphnia dubia*) in acute (48- to 96-hour) water exposures with 10 selected chemicals in Tier 1 testing, and to "screen" acute sensitivity to additional 10 chemicals with a commonly tested mussel species (fatmucket, *Lampsilis siliquoidea*) in Tier 2 testing. The mussels for Tier 1 testing were selected to be representative of five tribes of the family Unionidae widely distributed in the Midwest and the Southeast of the US, and the family Margaritiferidae in the Pacific Northwest. Two species of snails to be tested also have a wide geographic distribution in the US. The chemicals selected were chosen based on the AWQC of interest, availability of toxicity data for non-mollusks, other non-unionid mollusks sensitive to the chemical, and different toxic modes of action. Median effect concentrations for each chemical estimated from this study will be compared with those in current national databases for all freshwater species. The species mean acute values for all species will be ranked and plotted in cumulative distribution for each chemical to evaluate the sensitivity of mollusks. Results of this study could be used by the EPA and FWS to evaluate risks of contaminants to mussels or snails and in development or update of AWQC, state water quality standards, or permit limits.

**RP201 Age related contaminant dynamics in young bull sharks (*Carcharhinus leucas*)** J.A. Olin, M.C. Beaudry, G. Paterson, A.T. Fisk, Great Lakes Institute for Environmental Research. Persistent organic pollutants (POPs) including polychlorinated biphenyls (PCBs) and organochlorine (OC) pesticides pose an important anthropogenic risk to top predator species in aquatic food webs. Despite such concerns, few studies have investigated POP bioaccumulation kinetics in top predator fish species, especially for younger individuals that are particularly threatened by anthropogenic pollution because of their tendency to bioaccumulate and biomagnify environmental contaminants. In this study, young bull sharks (*Carcharhinus leucas*) were sampled from two subtropical estuarine river systems of southwestern Florida, the Caloosahatchee and Myakka, from 2006–2008. Individuals were assessed for age using umbilical scar stage (USS) identification, measured for total length, and liver samples were collected and analyzed for 37 PCB congener and 19 OC pesticide residue concentrations. Bull sharks ranged in total length from 69 – 151 cm which encompassed four USSs, in addition to individuals categorized as 1-2, and 2+ year old. Liver tissues averaged  $65 \pm 11\%$  lipid content (dw) with lipid normalized  $\Sigma$ PCB concentrations ranging from 315 – 6841 ng-g (lipid)<sup>-1</sup>. Detectable concentrations of all 37 PCB congeners were quantified in each sample. Organochlorine pesticide profiles were dominated by p,p'-DDE with concentrations ranging from 358 – 5050 ng-g(lipid)<sup>-1</sup>. Detectable levels of mirex, nonachlor, and chlordane compounds were also quantified in all samples. Normalization of PCB congener concentrations quantified in each age class to those of the youngest shark age class (USS2) demonstrated a significant hydrophobicity (Log *Kow*) effect. Specifically, the burdens of highly hydrophobic (Log *Kow*  $\geq 6.5$ ) compounds quantified in USS2 individuals were indicative of maternal offloading with half-lives to eliminate maternal PCB congeners estimated at up to 2 years. Following this period, exogenous feeding was considered to influence the POP profiles quantified in older individuals. This study is among the first to quantify patterns of POP bioaccumulation across juvenile shark age classes and demonstrates that the youngest life stages of this species has substantial burdens of POPs, likely a result of maternal offloading.

**RP202 Application of Incremental Sampling Methodology to Ecological Risk Assessment** R. Weil, L. Stuchal, University of Florida; L. Mora-Applegate, Florida Department of Environmental Protection; S. Roberts, University of Florida. Incremental sampling methodology (ISM) is a systematic sampling protocol based on the sampling theory of Pierre Gy. ISM has been developed in order to reduce data variability and provide a more accurate estimate of the mean concentration of an analyte across an area of concern. Proper implementation of ISM requires systematic planning, which includes defining the volume of the soil over which a management decision can be made (decision unit (DU)), development of an accurate conceptual site model (CSM), and purpose-driven sampling. ISM has a number of potential advantages over discrete sampling in characterizing site contamination for ecological risk assessment, especially when funding for site characterization is limited. This is because ISM is inherently more efficient than discrete sampling in providing a reliable estimate of the mean concentration within a specified area of concern (i.e., ISM can provide the information with fewer samples to analyze). Planning for ISM sampling



to support an ecological risk assessment can be complex, however. Consideration of multiple ecological receptors can entail selection of multiple overlapping DUs of different sizes to accommodate different home ranges. This results in more complicated field sampling work plans and more time in the field taking samples than is typically the case with traditional discrete sampling exercises. When properly planned, results from small DUs can be combined using weighted apportionment to estimate the mean for larger DUs, resulting in some additional reduction in analytical laboratory costs. ISM methodology can be adjusted for larger sites where contaminants are homogeneously distributed by extrapolating the coefficient of variation (CV) across DUs. Extrapolation requires some evidence of homogeneity and an assessment of the over- or underestimation of risk due to the uncertainties involved in extrapolation.

**RP203 Bioaccumulation and biomarker responses of *Hydrocynus vittatus* from two Rivers in the Kruger National Park, South Africa** R. Gerber, Centre for Aquatic Research, University of Johannesburg / Department of Zoology; V. Wepener, Centre for Aquatic Research, University of Johannesburg / Zoology, Rand Afrikaans University; N.J. Smit, School of Environmental Sciences and Development, Potchefstroom Campus, North West University / School of Environmental Sciences and Development. This study made use of the tigerfish, *Hydrocynus vittatus* as an indicator of changes in the water quality of two major Rivers flowing through the Kruger National Park, South Africa. The aim was to determine the spatial and temporal pollutant (metal and persistent organic pesticide) exposure in *H. vittatus* from the Olifants and Luvuvhu Rivers, and relate these exposures to their biomarker responses. Tissue samples for bioaccumulation and biomarker studies were collected during the period of September 2009 through to May 2011. Samples were collected during both low flow and high flow periods. Tissue bioaccumulation is used to relate environmental contaminants (metals and organic pollutants) levels in water and sediments to the bioavailable fraction in the muscle tissue of the tigerfish. Digestion and metal analysis in fish tissue were conducted using ICP-OES and ICP-MS techniques. Organic contaminant extraction (solid phase matrix dispersion) and analyses (GC-MS) were carried out using standardised techniques. The biochemical markers measured were: biomarkers of effects; Lipid peroxidase (LP), Catalase (CAT) activity, Protein carbonyls (PC) and cellular energy allocation (CEA) and biomarkers of exposure; Acetylcholinesterase (AChE), Cytochrome P450 activity (CYP450) and Metallothioneins (MT). The biomarkers of exposure for organophosphate pesticides (AChE and MT) were greater in the Olifants River when compared to the Luvuvhu River, whereas the organochlorine biomarker of exposure (CYP450) was more pronounced in the Luvuvhu River. The oxidative stress biomarkers reflected this exposure to organochlorides whereas protein damage was observed in the Olifants River fish. The biomarker responses are reflected through the exposure and subsequent bioaccumulation of predominantly organic pollutants in the Luvuvhu and metals in the Olifants Rivers.

**RP204 Chemical Dispersants Are Cytotoxic to Humpback Whale and Sperm Whale Skin Cells** L. Falank Jr., Wise Laboratory of Environmental and Genetic Toxicology, Maine Center for Toxicology and Environmental Health, Department of Applied Medical Science / Department of Applied Medical Science; C.F. Wise, J.P. Wise Sr, Wise Laboratory of Environmental and Genetic Toxicology, Maine Center for Toxicology and Environmental Health, Department of Applied Medical Science. Louis C. Falank, Jr., Catherine F. Wise, and John Pierce Wise, Sr. Wise Laboratory of Environmental and Genetic Toxicology, Maine Center for Toxicology and Environmental Health, Department of Applied Medical Science, 96 Falmouth St., Portland, ME 04104. Our research goal is to characterize the potential impact of dispersants and dispersed oil on marine life so that better policies and procedures can be developed to protect against future spills and to better understand ocean pollution. We are pursuing this goal by using whales as model marine species. As air breathing, warm-blooded mammals that nurse their young, whales and their marine mammal relatives represent humans most closely in the ocean. We are using whale cells to determine the impacts on whale DNA. We are testing the hypothesis that dispersants and dispersed oil can damage whale DNA. Part of this study is determining the toxicity of the two dispersants (Corexit® EC9500A and EC9527A); both of which were used in Alaska and the Gulf of Mexico. Our data show that both dispersants are toxic to humpback and sperm whale skin fibroblasts. Cells were treated with and without S9 fractions with cofactors, because fibroblast cells may not readily express cytochrome p450 enzymes that may help to

metabolize the chemicals. EC9500A was cytotoxic to humpback whale and sperm whale skin fibroblasts. In humpback whale cells, 50, 250, 500, and 1000 ppm, induced 87.4, 90.9, 57.7, and 14.6 percent relative survival, respectively. In sperm whale cells these doses induced 82.3, 67.1, 25.3, and 2.9 percent relative survival respectively. S9 fractions did not modify the effect in either species. EC9527A was also cytotoxic to humpback whale and sperm whale skin fibroblasts. In humpback whale cells 50, 250, 500, and 1000 ppm, induced 78.2, 79.6, 70.9, and 27.4 percent relative survival, respectively, while in sperm whale cells these doses induced 109.5, 108.3, 93.5, 37.2, 44.2 percent relative survival respectively. S9 fractions did increase the toxicity for the sperm whale cells, but did not modify the effect on the humpback whale cells. These data suggests that chemical dispersants should be considered an environmental risk for marine life. Ongoing and future work will consider the genotoxic effects of dispersants as well as the effects of dispersed oil on whale fibroblast cells.

**RP205 Clarifying Variability Within Longitudinal and Cross-Sectional PCB Body Burden Age Relationships in Long-Lived Marine Mammals** M. Binnington, University of Toronto Scarborough / Dept. of Physical & Enviro. Science, UTSC – Dept. Physical & Environmental Sciences; C.L. Quinn, University of Toronto Scarborough / Dept. of Physical & Enviro. Science; F. Wania, University of Toronto Scarborough / Dept. of Physical & Enviro. Science, University of Toronto at Scarborough / Dept. of Physical & Enviro. Science. Long-lived marine mammals are frequently used for the biomonitoring of persistent and bioaccumulative organic substances such as polychlorinated biphenyls (PCBs). When concentrations of such substances in sampled populations are measured, they are often plotted against age and interpreted as if they reveal information on the contamination of individuals as they age. However, as was recently shown for humans, cross-sectional body burden age trends (CBATs), which are obtained from biomonitoring studies that sample individuals of different age at the same time, should not be confused with longitudinal body burden age trends (LBATs), which apply to individuals sampled repeatedly over time. We used bioaccumulation models to calculate LBATs for PCB-153 in marine mammals (specifically Arctic ringed seals (*Pusa hispida*)) born at different times relative to time-variant historical environmental contamination. Model results from longitudinal PCB congener 153 exposure simulations for individual ringed seals were generated, and by repeatedly “sampling” these individuals it was further possible to construct CBATs for different years. These were then compared with measured CBATs for seal populations reported in the literature to evaluate the accuracy of model output. Both longitudinal and cross-sectional body burden age relationships in seal differed appreciably over time, with time from peak PCB-153 emissions being the most significant predictor of variability. In contrast to CBATs of PCB-153 in longer-lived humans, the CBATs of seal are always increasing. However, the model suggests that during times of increasing environmental contamination, the concentrations in older seal (30 to 40 years) are only marginally higher than in somewhat younger seals (20 to 30 years), whereas the older seals have markedly higher contamination if the population is sampled many years after the peak in emissions occurred. Despite their shorter lifespan, measured CBATs for seals cannot be interpreted as if they applied to individuals.

**RP206 Comparison of alternate strains of *Navicula pelliculosa* to replace strain 664 for ecotoxicological testing** K. Soucy, K. Softcheck, M. Staggs, Smithers Viscent. Major regulatory agencies (e.g., USEPA and OECD) provide guidance for conducting environmental toxicity tests with chemical substances and/or mixtures. Within their guidelines certain species and strains of the test organisms are recommended based on performance characteristics and the existing databases, for example the freshwater diatom *Navicula pelliculosa* strain 664, from the University of Texas. Recently, this strain became unavailable and alternate strains from different commercial suppliers have had to be considered. However, there is some uncertainty as to which strain of *N. pelliculosa* should be used as a replacement. The objective of this investigation was to evaluate several available strains of *N. pelliculosa* against a common reference toxicant and provide data on the relative sensitivity and growth performance of the alternate strains in comparison to strain 664.

**RP207 Cytochrome P4501A1 as a Biomarker of Contaminant Exposure in Stranded and Free-ranging Hawaiian Odontocetes** K. Foltz, S.F. White, Hawaii Pacific University / Natural sciences; R. Baird, Cascadia Research Collective; G. Ylitalo, Northwest Fisheries Science Center; B.

Jensen, Hawaii Pacific University / Natural sciences. Cytochrome P4501A1 (CYP1A1) is a biomarker used to assess the health of cetaceans exposed to agonists of the aryl hydrocarbon receptor (AHR), such as halogenated aromatic hydrocarbons (HAHs) and polycyclic aromatic hydrocarbons (PAHs). Endothelial cells are known to express CYP1A1 and are prevalent in the blubber of cetaceans. Stratification of CYP1A1 in the blubber may be intensified when AHR agonists are mobilized from adipocytes. Previous work with live-captured bottlenose dolphins showed CYP1A1 predominance in the deep blubber layer. Dart-biopsies of free-ranging animals in the wild, however, may only obtain the superficial to middle blubber layers. The present study assessed stratification of key cell types and CYP1A1 in full-depth blubber samples from stranded Hawaiian odontocetes ( $n=32$ ) to determine the utility of small biopsy samples for CYP1A1 analysis. Formalin-fixed blubber cross sections were examined histologically using standard H&E protocols. CYP1A1 expression was examined by immunohistochemistry using a polyclonal anti-CYP1A1 antibody, and was quantitatively measured based on staining occurrence and intensity. Factorial repeated measures ANOVA was used for analyses. CYP1A1 expression was significantly higher in the deep blubber layer compared to the middle and superficial blubber layers ( $p=0.013$ ). However, because deep blubber CYP1A1 expression significantly correlated with the middle and superficial layers ( $p=0.002$  and  $p=0.034$ ), it was concluded that biopsies could adequately represent full depth sections when scores are normalized to biopsy area. Body condition significantly influenced CYP1A1 expression throughout the blubber layers ( $p=0.047$ ), most likely because of contaminant mobilization. Using the same methods on dart-biopsy samples ( $n=113$ ), CYP1A1 expression was detected in ten species of free-ranging Hawaiian odontocetes. Species-specific CYP1A1 expression was detected ( $p=0.005$ ), but no differences were detected between gender or age class. Within false killer whale (*Pseudorca crassidens*) samples in which a suite of organic contaminants were measured,  $\Sigma$ PCBs wet weight (ng/g) correlated significantly with CYP1A1 expression ( $p=0.046$ ), yet suggested that expression in Hawaiian odontocetes is also influenced by other AHR agonists. Altogether, the results support the use of CYP1A1 as an indicator of contaminant effects in Hawaiian odontocetes.

**RP208 Effects of elevated PCO<sub>2</sub> on growth and development in the copepod *Calanus finmarchicus*** S.A. Pedersen, A.J. Olsen, Norwegian University of Science and Technology; D. Altin, BioTrix; I. Salaverria, Norwegian University of Science and Technology; B. Hansen, SINTEF Materials & Chemistry / Marine Environmental Technology. The high latitude copepod *Calanus finmarchicus* dominates the standing stock of zooplankton in the northern North Sea, Norwegian Sea and Barents Sea. Because of its central position in the food web, effects of elevated levels of CO<sub>2</sub> on this species due to ocean acidification or potential leakage from sub seabed storages may influence the whole ecosystem. Using a custom made microcosm system the development of egg to late copepodite stage was followed while exposed to different CO<sub>2</sub> levels (390, 3300, 7300 and 9700 ppm CO<sub>2</sub>). The one month experiment revealed significant reduction in survival at the two highest concentrations. Also, the relative proportion of developmental stages CIV and CV were significantly affected in a manner that indicate a CO<sub>2</sub> induced reduction of the ontogenic developmental speed. Morphometric analyses revealed significant increase in the size and lipid storage among CIV copepodites and a reduced size among stage III. The findings indicate that *C. finmarchicus* may be sensitive to CO<sub>2</sub>-levels that are within the range that may occur in association with a CO<sub>2</sub>-leakage episode from sub seabed storages.

**RP209 Effects of the fungal pathogen *Batrachochytrium dendrobatidis* and the pesticide endosulfan on Cascades frogs** D. Reagan, San Francisco State University / Department of Biology; C. Davidson, San Francisco State University; J. Piovio-Scott, D. Rejmanek, J. Foley, J. Worth, S. Lawler, University of California Davis; K. Pope, US Forest Service; C. Johnson, K. Aceituno, US Fish and Wildlife Service. In 2006, *Batrachochytrium dendrobatidis* (*Bd*), an emerging water-borne fungal pathogen was discovered in Cascades frogs (*Rana cascadae*) in California. In the southern Cascade Mountains, the Cascades frog was once common but is now found in only 10 small populations. The timing and speed of the decline coupled with the discovery of *Bd* in the remaining populations place *Bd* as a prime suspect for these declines. In contrast, Cascade frogs are still widespread in the nearby Trinity Alps Mountains, where *Bd* is also present. Environmental contaminants may alter disease susceptibility and could have contributed to the observed declines. Endosulfan, a current-use organochlorine pesticide, is the

most frequently detected chemical in tadpole samples from the California Cascades. We exposed juvenile Cascades frogs from the Southern Cascades and the Trinity Alps to one of two different strains of *Bd* isolated from Cascades frogs in California, endosulfan, or a combination of endosulfan and *Bd*. 91% (51 out of 56) of the frogs exposed to one of the *Bd* strains died within 40 days of exposure, while only 38% (18 of 47) of the frogs exposed to the other strain had died 97 days after exposure (compared to 17% of unexposed controls). Frogs that were exposed to endosulfan did not differ significantly from unexposed frogs in growth, mortality, or infection intensity. While we did not find an effect of endosulfan on disease susceptibility, we did find drastic differences in virulence between *Bd* strains collected from two separate Cascades frog populations, suggesting that *Bd* strain type influences the effect of *Bd* on Cascade frog populations.

**RP210 Environmental Risk Assessment of 4,5-Dichloro-2-n-octyl-3(2H)-isothiazolone for Wood Preservative Applications** J.P. Carbone, Dow Chemical Company / Toxicology, Environmental Research and Consulting, The Dow Chemical Company / Toxicology, Environmental Research and Consulting; J. Ryder, The Dow Chemical Company / Dow Microbial Control, Regulatory Affairs; G.A. Hazelton, The Dow Chemical Company / Toxicology, Environmental Research and Consulting. The environmental risk of 4,5-Dichloro-2-n-octyl-3(2H)-isothiazolone [DCOIT] to terrestrial mammals, avian species, terrestrial plants, soil dwelling microorganisms and macroinvertebrates, fish, aquatic invertebrates and algae was assessed based on the use of the biocide as a wood preservative. A qualitative assessment of the risk to companion animals from direct contact with treated lumber was also undertaken. The exposure analysis was conducted where wood preservation applications such as transmission poles, fence and deck posts, fences and decks and timber clad houses were utilized as the source of DCOIT entry into the environment. The USEPA simplified food web exposure model was employed for the terrestrial mammal and avian exposure analysis. Additionally, the USEPA's Express and SCI-GROW models were used to estimate environmental concentrations in surface water bodies and groundwater, respectively. Based on calculated risk quotient values using voles and bobwhite quail as representative species, the use of DCOIT as a wood preservative would pose a negligible risk to terrestrial wildlife and domestic mammals and avian species. DCOIT would not exert adverse effects on soil nitrification and mineralization process. Similarly, the probability of DCOIT adversely affecting terrestrial plant species is considered negligible. The potential of adverse effects to terrestrial macroinvertebrates such as the earthworm has also been shown to be negligible. With regard to aquatic organisms, the resulting calculated RQ values were consistently below the presumptive risk levels of concern. Experimental evidence indicates that DCOIT is not bioaccumulative. Considering the biodegradability of DCOIT metabolites and the reduced toxicity of the metabolites relative to parent and since the risk assessment demonstrates a negligible probability of adverse effects to organisms exposed to DCOIT the metabolites would have a similarly low probability of exerting adverse effects. Evaluation of the potential for groundwater contamination using USEPA SCI-GROW, indicated that the likelihood of DCOIT contamination of aquifers was negligible. The results of the risk assessment indicate that the use of DCOIT as a wood preservative would pose negligible adverse effects to the environment.

**RP211 Fathead minnow vs. zebrafish: A comparative analysis of development in two toxicologically relevant species** M. Bustos, A. Beasley, Middle Tennessee State University / Biology; R.R. Otter, Middle Tennessee State University / Biology, Middle Tennessee State University / Department of Biology, Middle Tennessee State University / Assistant Professor. Fathead minnow (*Pimephales promelas*) and zebrafish (*Danio rerio*) are two widely used, toxicologically relevant species. Fathead minnows are rarely utilized for studies aimed at investigation of teratogenic effects. Rather, zebrafish have been the focus of these studies due to well-defined stages of development and the transparency of their chorion. This study employed time-lapse microscopy to directly compare development in zebrafish and fathead minnow. Analysis of development showed very similar developmental processes occurring between these two fish species. Nearly all stages (< 90%) currently utilized for the description of zebrafish development were appropriate for the description of a developing fathead minnow. Furthermore, the embryo transparency and ease of handling make a strong case for the use of fathead minnow as a viable alternative to zebrafish in developmental studies.



**RP212 Freshwater snails exhibit a broad range of salinity tolerance:**

**increased invasion potential to higher – salinity environments** J.G. Suski, United States Environmental Protection Agency / Department of Biological Sciences; C.J. Salice, Texas Tech University / Environmental Toxicology, Texas Tech University / TIEHH, Texas Tech University / Department of Environmental Toxicology. *Physa pomilia* and *Helisoma trivolvis* are native freshwater snail species; however, their continuous reintroduction to the wild from the aquarium trade and rapid assisted spreading via wildlife has led to their establishment in nearly all freshwater systems. Interestingly, both species have been found in high-salinity environments ( $\sim 4000 \mu\text{Scm}^{-1}$ ) indicating they have a broad range of salinity tolerance. This study examined salinity tolerance of two populations of *P. pomilia* and *H. trivolvis*, those collected from a high-salinity environment (Tahoka, TX) and a low-salinity environment (Waco, TX). Snails were collected from their respective ponds and brought back to the laboratory and allowed to reproduce. Salinity tolerance was assessed by using egg masses from the field collected individuals; egg masses were evaluated for time to hatch and hatching success at 300, 600, 1200, 2400, 4800 and  $9600 \mu\text{Scm}^{-1}$  using NaCl to increased salinity. Results showed differences in both species – specific and population – specific response to a range of salinities. *H. trivolvis* hatched more rapidly at lower and moderate salinities when compared to *P. pomilia* regardless of population. Furthermore, *P. pomilia* and *H. trivolvis* egg masses from the high-salinity population hatched more rapidly than those from the low salinity at concentrations of 300, 600, 1200 and  $2400 \mu\text{Scm}^{-1}$ . Hatching success in both species and population was significantly decreased in high salinity ( $\geq 4800 \mu\text{Scm}^{-1}$ ). Overall, hatching success was significantly greater in *P. pomilia* egg masses from the high-salinity population at 300, 600 and  $1200 \mu\text{Scm}^{-1}$ , while there was no difference at salinities  $\geq 2400 \mu\text{Scm}^{-1}$  when compared to *P. pomilia* low-salinity population. Additionally, there was no difference in hatching success of *H. trivolvis* between either the high-salinity and low-salinity populations. These data provide insight into the broad range of salinity tolerance of two species of freshwater snails. *P. pomilia* hatchlings from the high-salinity population appear to exhibit a gain in acceptable ranges of salinity relative to those in the low-salinity population. Although there is still a threshold ( $9600 \mu\text{Scm}^{-1}$ ) *P. pomilia* and *H. trivolvis* may display enough phenotypic plasticity to successfully invade brackish habitats.

**RP213 Holistic Method of Developing Protective Ecological Risk-based Soil Concentrations for Cleanup Decisions**

J. Holder, Geosyntec Consultants, ERM; G.C. Weber, ERM; M. Pound, NAVFAC SW. Risk-based concentrations (RBCs) were developed for soil as removal goals for the time-critical removal action (TCRA) at Installation Restoration (IR) Program Site 1, Naval Auxiliary Landing Field (NALF) San Clemente Island. The RBCs are protective concentrations in soil that result in acceptable risk to the receptor groups of interest. These RBCs were used to delineate the removal area for the TCRA. RBCs were developed for a number of constituents of concern (COCs) (e.g., cadmium, copper, lead, zinc and PCBs), and for a variety of receptors (e.g. plants, invertebrates, avian and mammalian wildlife, and humans). A range of protective soil concentrations was then developed for each COC and receptor of interest, and RBCs were then selected as the removal goals for the TCRA. Selection of the RBC was not always based on the lowest value calculated. Instead, a holistic evaluation of the range of values, level of protectiveness for each receptor of interest, and professional judgment were used to select a value that was reasonably protective, without being overly conservative. RBCs were then compared to the range of background to ensure that they would not result in the removal of soils with concentrations that are naturally occurring. For this project, the purpose of the RBCs was to ensure that the removal area was adequately delineated; therefore, overly conservative RBCs were not beneficial as they could result in over excavation. To confirm the protectiveness of the removal action to human and ecological receptors, a residual risk assessment was conducted with the confirmation soil data collected during the cleanup. This presentation will discuss the methodologies used to develop RBCs and the outcome of the remedial action.

**RP214 Influence of stressor tolerance and reproductive mode on the invasive potential of the non-native snail *Biomphalaria glabrata***

M.A. Funkhouser, Texas Tech University / TIEHH, Texas Tech University; S. Plautz, Texas Tech University / The institute of environmental and human health, Texas Tech University; C.J. Salice, Texas Tech University / Environmental Toxicology, Texas Tech University / TIEHH, Texas Tech University

/ Department of Environmental Toxicology. Like chemical contaminants, invasive species can be responsible for considerable damage to natural resources. Despite the fact that contaminants and non-native species may frequently co-occur, there has been little research on stress tolerance traits of potential invaders. Many factors can impact whether a non-native species can become a potent invader including life history traits and stressor tolerance. We were interested in whether a non-native species displayed greater tolerance to environmental stressors than a native species with a similar life history and habitat requirement. We raised two, hermaphroditic freshwater pulmonate snail species, a native wild-caught snail (*Physa pomilia*) and a non-native snail from laboratory cultures (*Biomphalaria glabrata*), either singly or in groups to monitor species differences and the impact of reproductive mode (self-fertilization or outcrossing). The native, *P. pomilia*, had lower survival than *B. glabrata* ( $P \leq 0.001$ ) under our laboratory conditions. *B. glabrata* produced more egg masses per snail per day ( $P \leq 0.001$ ), but *P. pomilia* produced more eggs per egg mass ( $P \leq 0.001$ ). While individual *B. glabrata* laid more egg masses per snail per day than snails housed with conspecifics, individual *P. pomilia* laid less than multiples. *B. glabrata* reproduced before *P. pomilia* ( $P \leq 0.001$ ) and snails reared in groups started reproducing before those raised individually ( $P \leq 0.001$ ). Being housed with conspecifics made no difference in the size of *B. glabrata* ( $P = 0.9567$ ), but single *P. pomilia* were smaller than those reared with conspecifics ( $P = 0.0145$ ). The offspring of *P. pomilia* were more tolerant than those of *B. glabrata* to salinity and cold, but less tolerant of cadmium, heat, and malathion ( $P \leq 0.05$ ). Offspring from self-fertilization were less tolerant of all stressors than offspring of outcrossed snails ( $P \leq 0.05$ ). Overall, native, wild *P. pomilia* did more poorly than the non-native *B. glabrata* when alone, as may happen in the colonization of a new area. The invasive potential of *B. glabrata* as compared to the native *P. pomilia* is seen by the shorter time to reproduction, greater reproductive output, and greater tolerance to certain stressors. Also, data here suggest that chemical contamination may predispose certain habitats toward successful establishment of non-native species.

**RP215 Influence of temperature on developmental rate of fathead minnows using morphological endpoints**

A. Beasley, Middle Tennessee State University / Biology; R.R. Otter, Middle Tennessee State University / Biology, Middle Tennessee State University / Department of Biology, Middle Tennessee State University / Assistant Professor. Fathead minnows (*Pimephales promelas*) are extensively used in toxicological research and regulatory testing, particularly in North America. Despite such broad application, fathead minnows are rarely chosen as a model organism for developmental studies. Here we evaluated the influence of temperature ( $24.5\text{--}28.5^\circ\text{C}$ ) on several morphological endpoints: developmental staging, ear-eye distance, and ratio of ear-eye distance to inner ear diameter. As temperature decreased, all endpoints revealed delays in development, with differences between endpoints in amount and type of delay measured. Integrated predictive equations were created to normalize each endpoint for temperature. Similarities between stages of development in fathead minnow and zebrafish (*Danio rerio*) highlight the potential of fathead minnows as a model organism for future developmental studies.

**RP216 Influence of Total Dissolved Solids from Natural Resource Extraction Activities on *Prymnesium parvum* Growth and Toxicity**

K.N. Prosser, C. Scott, S.A. Eytcheson, Baylor University; J. Lazorchak, C. Neitich, U.S. Environmental Protection Agency; B. Brooks, Baylor University. *Prymnesium parvum* harmful algal blooms (HABs) have become an emerging threat to inland freshwater systems due to the salinization of surface waters resulting from anthropogenic influences such as natural resource extraction activities (NRE) including hydraulic fracturing (HF) and mountaintop mining (MTM). Previous research from our team has identified salinity as being a critical parameter influencing *P. parvum* HAB dynamics in inland waters; however, the effect of site-specific ionic constituents is not well understood. Elevated site-specific total dissolved solid (TDS) concentrations of ionic constituents associated with these practices may increase the threat of *P. parvum* to inland waters by affecting HAB distribution, formation, and toxicity. In this study, we assessed the effects of TDS concentrations associated with HF and MTM practices on *P. parvum* HAB dynamics. Treatment levels of environmentally relevant HF and MTM contaminants were selected at varying TDS concentrations (HF: 130, 500, 2000, 4000, 8000 mg/L; MTM: 130, 500, 1000, 4000, 8000 mg/L), respectively. An additional experiment was designed to assess the effect of HF contaminants at varying TDS concentrations (130, 1000, 4000, 8000 mg/L) and various levels of



nutrients (F/2, F/4, F/8) on *P. parvum* growth and toxicity to *Pimephales promelas*. *Prymnesium parvum* cell densities were monitored throughout each study and exponential growth rates calculated. Results showed that increased ionic constituents associated with HF NRE stimulated *P. parvum* growth in a dose dependent manner with the maximum densities occurring at the 8000 mg/L TDS treatment level. Conversely, ionic constituents associated with MTM NRE did not stimulate *P. parvum* growth at any treatment level. Future studies are necessary to develop predictive models of environmental conditions leading to harmful *P. parvum* HABs in regions experiencing intensive NRE.

**RP217 Metal Accumulation from Dietary Exposure in the Sea Hare, *Aplysia californica*** T.A. Jarvis, Valdosta State University / Department of Biology; T. Capo, University of Miami; G. Bielmyer, Valdosta State University / Department of Biology. Marine ecosystems are heavily influenced by metals due mainly to anthropogenic sources. Several metals often co-occur at metal-impacted sites in the environment. Recent studies have reported the significance of dietary metal transfer in aquatic food chains, particularly in lower trophic levels. This study investigated the accumulation and effects of dietary metals in a macro invertebrate. In this study, the green seaweed, *Ulva lactuca* and the red seaweed, *Agardhiella subulata* were each concurrently exposed to three concentrations (10, 100, 1000 mg/L) of five metals (Cu, Ni, Pb, Cd, and Zn) for 48 h and then the seaweed species were used as diets for the sea hare, *Aplysia californica* for two weeks. Body mass and length of *A. californica* were measured midway and at the end of the exposure duration. The sea hares were then dissected and their organs were digested and analyzed for metals. The results demonstrated that metal accumulation varied with different metal species. Each metal used accumulated within at least one organ, with Cu accumulation being the greatest. In most of the organs analyzed, there were higher levels of metals within *A. californica* fed *U. lactuca* as compared to *A. subulata*. These results indicate that *U. lactuca* may accumulate metals in a more bioavailable form than within *A. subulata* and/or that *U. lactuca* may be a more nutritious diet than *A. subulata*. Significantly reduced body length and weight were observed between the control and metal-exposed *A. californica* at the end of the exposure period. This research supports the use of *A. californica* as a bioindicator of metal pollution in aquatic systems.

**RP218 Relationships between cytochrome P450 activity and allelochemical toxicity in butterflyfish (*Chaetodon* spp.) of differing feeding strategies** A. Maldonado, University of California, Riverside / Environmental Sciences, University of California, Riverside; R. Lavado, University of California Riverside / Department of Environmental Sciences, University of California Riverside / Department of Environmental Science; D. Schlenk, University of California-Riverside / Department of Environmental Sciences; S. Knutsen, G. Ostrander, University of Hawaii at Manoa; M. Slattery, S. Ankisetty, University of Mississippi. Cytochrome P450 monooxygenase (CYP) is the primary enzyme system for detoxification of xenobiotics including dietary chemicals and pollutants. Some herbivorous insects have the ability to detoxify toxic dietary chemicals by specific CYP forms. While thorough research has uncovered certain relationships between herbivorous insects and dietary chemicals, little is known about biotransformation and detoxification of allelochemicals derived from dietary products in marine organisms. Certain species of butterflyfish of the genus *Chaetodon* have been shown to feed on several species of chemically-defended corals including the soft coral *Simularia*. This study examined the effects of the natural product, 5-episinuleptolide (5ESL) on the expression and catalytic activities of CYP3A and CYP2 in two butterflyfish species one of which is an obligate coral feeder and a generalist feeder that can feed on coral. Fish were gavage 1.0 mg/kg and 3.0 mg/kg of 5ESL. Initial mortality indicated that *C. multicinctus* (obligate hard coral feeder), had 100% mortality in both doses. In contrast, 80% survival was observed in *C. auriga* (generalist feeder). Testosterone hydroxylase (TOH) (6 beta, 16 alpha, 16 beta) was 130-740 times lower in *C. multicinctus* relative to *C. auriga*. These results indicate an association between CYP2 (16-alpha TOH) and CYP3A (6 beta, 16 beta TOH) catalytic activities and detoxification of 5ESL in butterflyfish with different feeding strategies which may provide a selective advantage in allowing generalists to feed on chemically defended prey.

**RP219 Sensitivity of pulmonate and non-pulmonate snails in chronic toxicity tests with copper and ammonia** R.A. Consbrock, U.S. Geological Survey / Columbia Environmental Research Center; J. Besser, U.S.

Geological Survey / Columbia Environmental Research Center, USGS / Columbia Environmental Research Center; C.G. Ingersoll, USGS / Columbia Environmental Research Center, U.S. Geological Survey / Columbia Environmental Research Center; D.K. Hardesty, C.R. Warbritton, U.S. Geological Survey / Columbia Environmental Research Center. Most toxicity tests with freshwater snails have been conducted with few genera of abundant and easily-cultured pulmonate snails (e.g. *Lymnaea*, *Physa*). It is unclear whether results of these tests adequately represent the sensitivity of non-pulmonate snails, which make up a greater proportion of snail taxonomic diversity and are more frequently listed under the Endangered Species Act (ESA). Previous studies in our laboratory suggested the pulmonate snails may be less sensitive to the toxicity of ammonia and copper than non-pulmonate snails in the diverse family Hydrobiidae. We compared the sensitivity of these two groups to ammonia and copper based on tests with two pulmonate snails (*Lymnaea stagnalis* and *Physa gyrina*) and four Hydrobiid snails ('Idaho springsnail', *Pyrgulopsis robusta*, Bliss Rapids snail, *Taylorconcha serpenticola*; an unnamed pebblesnail, *Fluminicola* sp.; and Ozark springsnail, *Fontigens aldrichi*). Chronic (28-d) flow-through toxicity tests were conducted in proportional diluters with hard reconstituted water (hardness 160 to 180 mg/l; dissolved organic carbon about 0.5 mg/L) at 20 °C. Test waters of ammonia tests were maintained at pH 8.2 using automated pH controllers. Tests with *Lymnaea*, *Physa*, *Pyrgulopsis* and *Fluminicola* were started with uniform-age cohorts and included survival and growth endpoints; other tests were conducted with mixed ages and survival was the only endpoint. Results of these tests indicated that all 4 Hydrobiid taxa were more sensitive to ammonia than *Lymnaea* or *Physa*. Lowest ammonia LC50s (expressed as total ammonia) from tests with hydrobiids ranged from 0.93 mg N/L to 5.6 mg N/L, whereas both *Lymnaea* and *Physa* had LC50s greater than 8 mg N/L, the highest nominal concentration tested. Copper LC50s from tests with pulmonate snails were also greater (20-30 µg/L) than those for hydrobiids (12-23 µg/L), but growth of both pulmonates was reduced at lower copper concentrations (12-16 µg/L). None of the hydrobiids had significant reductions in growth in copper tests, perhaps reflecting their slower growth rates. These results suggest that the toxicant sensitivity ESA-listed snails of the family Hydrobiidae is better represented by testing with surrogate hydrobiid taxa than by tests with easily-cultured pulmonate snails. However, laboratory culture and toxicity testing of hydrobiid snails can be limited by long generation times, low reproductive output, and slow growth rates.

**RP220 Terrestrial Ecological Risk Assessment of Fugitive Dusts from Tailings from a Large Zinc Mine** C. Moore, Intrinsik Environmental Sciences Inc.; L.J. Marshall, Intrinsik Environmental Sciences / Environmental Scientist; S. Rodney, Intrinsik Environmental Sciences Inc.. A large zinc mine located in Eastern Canada has been operating since the mid-1960s. Over the years of operations, fugitive dusts from their tailings pile have lifted off and dispersed through adjacent forested areas. While mitigative measures have been implemented to reduce these occurrences, releases during earlier years needed assessment to determine if risks to the environment were elevated. An ecological risk assessment was conducted using a sequential approach. The approach included initial characterization of contaminants in soils in areas adjacent to the mine, as well as several distant reference locations, to determine the size of area affected by fugitive dusting events. Characterization of metals in other parts of the ecosystem, including soil invertebrates, small mammals and vegetation, was conducted, as well as food chain modeling to estimate risk levels in various receptor groups. Additional lines of evidence, such as a breeding bird survey, vegetation community assessment, vegetation growth trials, and tissue residue data from selected organs from two receptors, were used to characterize risk. The results of the study indicated that windblown tailings have affected soils in the south and south-easterly direction of the mine site. Terrestrial vegetation communities immediately south of the mine site have been impacted by mine site releases. There is a high potential for community level effects in soil invertebrate and soil microorganism communities in near-field areas south of the mine. Effects on vegetation and soil invertebrate communities decrease with increased distance from the mine. It is considered unlikely that metals in soils, surface waters or foods are having a direct effect on terrestrial wildlife populations foraging near the mine site. Secondary effects could be occurring in some wildlife species, related to alterations in habitat, and/or nesting materials or food availability issues related to poor soil conditions and elevated metals concentrations. The approach and outcomes of the assessment will be presented, as well as the path forward.

**RP221 The effect of Acid Mine Drainage on the hatching success of Branchiopod eggs** M. Ferreira, Centre for Aquatic Research, University of Johannesburg / Zoology Dept.; W. Malherbe, Centre for Aquatic Research, University of Johannesburg / Zoology Department, Centre for Aquatic Research, University of Johannesburg / Department of Zoology; V. WEPENER, J. van Vuren, A. Henri, Centre for Aquatic Research, University of Johannesburg / Department of Zoology. Pans are endorheic wetlands, and in South Africa many of these wetlands occur in areas of increasing mining activity. Many of these systems are already affected by acid mine drainage (AMD) and an increase in energy demand has caused an increase in mining activities surrounding these wetlands. The potential effect of AMD on the hatching success of Branchiopod eggs is of particular concern. These wetland ecosystems are highly unpredictable, with physical and chemical conditions changing as these systems change from a dry to a wet phase and between different wet phases. Branchiopod crustaceans survive this unpredictability by having a dormant egg phase that is desiccation resistant. The effect of AMD on the hatching success of egg banks has not been well studied locally or internationally. The aim of this study is thus to contribute to our knowledge of the effect of water quality changes on the branchiopod communities within these systems. For the purpose of the study, 30 pans were included and five core samples were collected from each pan in a transect moving from the perimeter towards the centre of the pan. The content of each core sample was combined to create a composite sample of each pan. The composite was mixed and divided to create three subsamples for each pan. After an incubation period, each of the sub samples was transferred to experimental aquaria and treated with different solutions including reconstituted water with salinity adjusted to 1000 mg/l, 1500 mg/l, and AMD collected from selected decant sites. The environmental room was kept at a constant temperature and light regime and Branchiopods was sampled every fourth day. It is expected that no eggs will hatch from the sediment samples exposed to AMD. Should no hatching take place from the aquariums that contain AMD, the AMD will be drained and replaced with adjusted reconstituted water at the end of the experiment. The physico-chemical variables of the water in each aquarium will be monitored during every sampling occasion and adjusted if necessary. The hatching success of each of the exposures will be compared by using a range of multivariate and univariate statistical analysis. The results of the study will contribute to the knowledge of the effect of mining related pollutants on the hatching success of Branchiopod communities. The knowledge is very important for future recommendations regarding the management and the protection of these ecosystems.

**RP222 The impact of temperature on the partitioning of TNT, RDX and triazine pesticides between Eastern oyster larvae and the marine environment** K. Raub, P. Vlahos, University of Connecticut. The purpose of this study is to investigate the impact of temperature on the partitioning of target munitions compounds and the Eastern oyster larvae, *Crasostrea virginica*. We examined the partitioning of 2,4,6-Trinitrotoluene (TNT), 1,3,5-trinitro-1,3,5-triazine (RDX), and the triazine based pesticides atrazine and terbutryn between seawater and the larvae. These compounds were chosen because they are structurally similar, three are triazine compounds, and they represent a range of  $\log K_{ow}$  values; 0.86, 1.86, 2.65 and 3.66 respectively. Eight day old larvae were incubated under three different temperature conditions (10°C, 18°C and 25°C) in media containing four different compound concentrations (12 treatments total, three replicates per treatment). The larvae were fed daily with *Isocrysis galbana* (T-ISO) grown at the respective compound concentrations. The oyster larvae were incubated for five days with water samples taken once daily prior to feeding to generate a time series of contaminant uptake and to compare between the various temperature conditions. On day five, final contaminant concentrations were extracted from the water, phytoplankton, and oysters and compared to initial values. Experimental uptake rates and partition coefficients were determined and compared to theoretical values.

**RP223 Toxicity and body burden of binary metal mixtures of copper, manganese and nickel to *Paronychiurus kimi* (Collembola)** Y. Lee, Korea University / Division of Environmental Science and Ecological Engineering, College of Life and Environmental Sciences, Korea University / Environmental Science and Ecological Engineering; J. Son, University of Nebraska-Lincoln / School of Biological Sciences; Y. Kim, Korea University / Division of Environmental Science and Ecological Engineering, College of Life and Environmental Sciences; S. Yu, Korea Univ.; K. Cho, Korea University / Division of Environmental Science and Ecological Engineering,

College of Life and Environmental Sciences, Korea University / # 407, Division of Environmental. In soil ecosystem, chemicals are often found as mixtures. Therefore, the toxicity data generated by single toxicity tests are inadequate for assessing the potential risk of complex mixture of chemicals for soil ecosystems. In the present study, the toxicity of binary metal mixtures of copper (Cu), manganese (Mn) and nickel (Ni) in soil to the reproduction of *Paronychiurus kimi* was evaluated using a toxic unit (TU) approach (1TU=EC50). In addition internal body concentrations of *P. kimi* after 28-days exposure at all combination of the metal mixture and each single of the metal in soil were measured. The 28-d EC50 mixture was 0.82, 0.87, and 1.03 TU for Cu-Mn, Cu-Ni, and Mn-Ni, respectively and internal body concentrations of *P. kimi* which was exposed at combination of the metal mixture were different from that which was exposed at single metal treated soil. These results indicate that the toxicity of metal mixtures in soil is substantially different from that of expected on the basis of additivity of individual metals due to interactions between metals in mixture. Therefore, these interactions affect on metal flowing in their body and the toxicity.

**RP224 Water Quality Effects on Survival, Growth, and Feeding Performance in the Federally Listed Delta Smelt (*Hypomesus transpacificus*)** K. Aceituno, U.S. Fish and Wildlife Service / Fish and Wildlife Biologist, U.S. Fish and Wildlife Service / Environmental Contaminants Division; S. Foott, U.S. Fish and Wildlife Service / California-Nevada Fish Health Center; C. Johnson, U.S. Fish and Wildlife Service / Environmental Contaminants Division. The Sacramento-San Joaquin Delta (Delta) is a complex system of tidally-influenced, interconnected waterways that serves as a vital resource to both humans and wildlife. In recent years dramatic population collapses have been observed in a number of fish species, including the federally threatened delta smelt (*Hypomesus transpacificus*). A long history of industrial, agricultural and urban land use in the Delta has led to extensive environmental impacts from the release of anthropogenic contaminants, which have been widely recognized as a factor contributing to recent declines. However, contaminant impacts on delta smelt population dynamics remain poorly understood. The primary objective of this investigation is to assess sub-lethal, pathological and physiological stresses imposed on larval delta smelt by water-borne contaminants in the Delta. Bioassays were conducted using delta smelt eggs obtained from the Livingston Stone National Fish Hatchery's refugial population program. Eggs and larvae were reared in Delta water, and post-hatch survival, growth rate and feeding performance were monitored. During water collection for the bioassay tests additional water samples were collected for contaminant analysis. Samples underwent pyrethroid, organophosphate, organochlorine, and fungicide analysis to determine if sub-lethal impacts are correlated to concentrations of contaminants detected in water from the Delta. Although contaminant concentrations in the Delta may not be acutely toxic to delta smelt, they have the potential to elicit a host of sub-lethal effects that may be impairing growth, survival and reproduction. Assessing these sub-lethal effects will lead to a better understanding of the role contaminants play in recent population collapses.

**RP225 Wild Male Bluegill Legacy Contaminant Profile: Associated Reproductive Effects and Implications** J.J. Hillis, Southern Illinois University Carbondale / Fisheries and Illinois Aquaculture Center and Department of Zoology, Southern Illinois University-Carbondale / Fisheries and Illinois Aquaculture Center; M. Lydy, Southern Illinois University Carbondale / Fisheries and Illinois Aquaculture Center and Department of Zoology, Southern Illinois University-Carbondale / Fisheries and Illinois Aquaculture; J. Garvey, Southern Illinois University-Carbondale / Fisheries and Illinois Aquaculture Center. Endocrine disrupting chemicals in wild fishes could potentially disrupt reproduction by causing intersex. Legacy contaminants, specifically polychlorinated biphenyls and organochlorine pesticides, have been shown to be estrogenic or anti-androgenic likely contributing to intersex in males. These contaminants continue to persist in the environment despite being restricted from use in the United States. Many lakes where fishing is an important source of recreation have elevated contaminant concentrations. Bluegills *Lepomis macrochirus* play a crucial role in lake food webs as forage and sport fish and may be affected by legacy contaminants. We quantified the concentration of legacy contaminants in male bluegills, and paired these fish with females with below reporting limit concentrations of contaminants in research ponds to measure if there was an effect on reproductive output. We also evaluated male bluegills (N=200) for intersex. Lake specific bluegill tissue contaminant concentrations ranged widely



from below reporting limit to 2 ppm (non-lipid normalized basis) and were significantly different (Tukey's HSD,  $p < 0.05$ ), allowing for comparison. No intersex fish were observed. Young production per pond was reduced in a dose-dependent manner. The highest male contaminant treatment produced the least young in the pond study, which suggested alterations to reproductive behavior or viability of offspring.

**RP226 Acute and Chronic Effects of Decreasing pH (Acid Precipitation) on Frog Species From the Kruger National Park, South Africa** C. Farquharson, University of Johannesburg / Department of Zoology; V. Wepener, Centre for Aquatic Research, University of Johannesburg / Zoology, Rand Afrikaans University; W. Vlok, BioAssets. Frogs are found worldwide, from the equator to polar areas, probably in every conceivable environment but the most inhospitable. Their extreme sensitivity to changes in temperatures, precipitation and ultraviolet radiation, make them excellent "bioindicators" of environmental health. Global declines of amphibian populations have become an increasing concern. Shockingly, some cases report entire species are threatened to become extinct, even in areas thought to be protected. As South Africa's premier conservation area, the Kruger National Park has a duty to conserve and manage all indigenous wildlife, vegetation etc. occurring in the region. There has been concern in recent years over possible negative influences of acid rain on the Park's amphibian populations. With the aim of determining effects of decreasing pH on the biology of these amphibians, acute ("LC<sub>50</sub>"-type tests) and chronic acid tolerance bioassays were conducted on tadpoles of four frog species found in the park, i.e. *Chiromantis xerampelina* (Southern Foam Nest Frog), *Pyxicephalus edulis* (African Bullfrog), *Amietophrynus maculatus* (Flat-backed Toad) and *Hildebrandtia ornata* (Ornate Frog), using survival, deformities and growth as endpoints. The Southern Foam Nest Frog is the only species from the genus *Chiromantis* occurring in South Africa. The pH-values used for acute exposures were pH 6, 5.5, 5, 4.5, 4, 3.5 and 3. A negative control consisting of pond water collected from sources within the Kruger Park ( $\pm$ pH 7) and a positive control (1mg/L CdCl<sub>2</sub> at pH 3.5) were included. Chronic exposure pH-values were selected based on the results of the acute assays. Mortality observations and pH-stabilization took place every 24 hours. Both exposure assays were carried out in triplicate for all 4 species. "LC<sub>50</sub>" pH-values were calculated with the Trimmed Spearman-Kärber Method, which were 4.07, 4.55, 3.75 and 3.747 for *C. xerampelina*, *P. edulis*, *A. maculatus* and *H. ornata* respectively. For chronic exposures tadpole growth rate as well as size decreased with decreasing pH-levels, whereas tadpole deformities increased with decreasing pH-levels. This study will contribute to future management and conservation efforts of the Kruger National Park as well as maintaining the Park as a premier conservation area.

**RP227 Assessing Biochemical Changes in the African Toad (*Bufo regularis*) Exposed to Sublethal Concentrations of Endosulfan Pesticide** L. Tongo, University of Benin / Animal and Environmental Biology; L.I. Ezemonye, University of Benin / Zoology Dept. The sublethal effects of Endosulfan on Cortisol secretion, Acetylcholinesterase (AChE), Glucose, Glycogen and Total protein levels were assessed in the serum, brain, liver, lungs and gastrointestinal tract (GIT) of the adult African toad, *Bufo regularis*. Tissue bioaccumulation was also carried out to support data. Toads were exposed to endosulfan concentrations of 0.01, 0.02, 0.03 and 0.04 mg/l for 28 days. Cortisol and Total Protein levels in the tissues decreased compared to the control. The pesticide caused differential increase in glucose levels in the tissues with a simultaneous reduction in liver glycogen level. No clear trend was however observed for Acetylcholinesterase (AChE) levels. Accumulation results obtained showed accumulation in the tissues (Liver>Serum>Brain>Lung>GIT), with a direct relationship between tissue concentration and changes in the biochemical indices. The alterations in all the indices were significantly concentration dependent. The present study provided evidence that biochemical parameters were altered as a result of exposure to endosulfan pesticide. Cortisol, Glucose, Glycogen and Total protein levels could therefore be used as non-specific biomarkers in evaluating the effect of endosulfan.

**RP228 Behavior of Ghost Shrimp Exposed to Imidacloprid in the Laboratory: Implications for the Control of Burrowing Shrimp in Willapa Bay, Washington** A.T. Troiano, Washington Cooperative Fish and Wildlife Research Unit / School of Aquatic and Fishery Sciences, University of Washington, University of Washington / School of Aquatic and Fishery Sciences, University of Washington; J.M. Grassley, Washington Cooperative Fish and

Wildlife Research Unit, University of Washington; J.A. Frew, Washington Cooperative Fish and Wildlife Research Unit, University of Washington / School of Aquatic and Fishery Sciences, University of Washington / School of Aquatic and Fishery Sciences, University of Washington, Washington Cooperative Fish and Wildlife Research Unit / School of Aquatic and Fishery Sciences; A.T. Troiano, Washington Cooperative Fish and Wildlife Research Unit, University of Washington / School of Aquatic and Fishery Sciences. Imidacloprid (IMI) may be a viable alternative to carbaryl for controlling burrowing shrimp that destabilize sediments, resulting in poor survival and low yields of Pacific oysters (*Crassostrea gigas*). However, efficacy varies among substrates and laboratory tests indicate the shrimp are relatively insensitive to IMI with 24-h LC<sub>50</sub>'s > 12,000 ppb active ingredient [ai] and 10x greater than concentrations reported in sediment pore water following application of 4x the targeted application rate. These results contrast sharply with observed efficacy in the field, with significant mortality occurring 72-96 h post application in sandy substrate at 0.5 lbs ai/ac. Reasons are not known, but an understanding of the factors governing efficacy in the field may improve control within muddy substrates and those with eelgrass. That ESA-listed green sturgeon (*Acipenser medirostris*) appear to preferentially feed on IMI-exposed shrimp increases the importance. We exposed adult ghost shrimp (*Neotrypaea californiensis*) in seawater to IMI (Nuprid® 2F) simulating concentrations reported in sandy substrate following application of 0.5 lbs ai per acre. Concentrations were the means from a pore-water fate curve: 0-6 h = 150 ppb ai, 6-24 h (24), 24-48 h (10), 48-72 h (6), and 72-96 h (4.3) plus negative controls with 10 shrimp at each time interval (5 IMI, 5 control), a loading of 0.31 L/g, and water exchanges at the end of each time period. Water quality and overt effects were monitored. At the end of each exposure, the ability of shrimp to burrow in sandy substrate was evaluated. All IMI-exposed shrimp survived, were overtly affected, and none burrowed. In contrast, all controls appeared normal and 96% exhibited burrowing behavior. Results suggest applications of IMI (0.5 lbs ai/ac) will not directly kill shrimp, exposed shrimp may not be able to maintain their burrows, and death may result from burrow collapse. Factors that maintain burrow integrity (mud/organic matter, eelgrass roots) may explain differences in observed efficacy. "Sublethal" overt effects may also increase detectability of IMI-poisoned shrimp by green sturgeon. Laboratory tests with shrimp exposed to IMI within burrows are needed.

**RP229 Sensitivity of White Sturgeon (*Acipenser transmontanus*) to Selected Environmental Pollutants** D.W. Yardy, University of Saskatchewan / Toxicology Centre, University of Saskatchewan / Ph.D candidate, University of Saskatchewan / Toxicology; J. Doering, S. Beitel, B. Tendler, University of Saskatchewan / Toxicology; J.P. Giesy, University of Saskatchewan / Biomedical Veterinary Sciences & Toxicology Centre, Michigan State University / Department of Animal Science, University of Saskatchewan / Toxicology Centre; M. Hecker, University of Saskatchewan / Toxicology Centre. Populations of sturgeons (Acipenseridae) are threatened throughout the world and numbers of individuals in North America, Asia, and Northern Europe have been decreasing over the past century. In North America, populations of white sturgeon (WS; *Acipenser transmontanus*) are declining, primarily due to poor annual recruitment, and have been listed as endangered in parts of north-western USA and British Columbia, Canada. Pollution has been hypothesized as one potential cause for poor recruitment in the Columbia, Fraser, and Sacramento-San Joaquin rivers and their tributaries. Specifically, there are concerns about the potential toxicity to WS early life stages (ELS's); a period of development when fish are considered to be most sensitive to the exposure of contaminants. WS live in close proximity to sediments, and thus, may be at particular risk from effects of pollutants associated with sediments. Little is known about the potential toxicity of inorganic and organic pollutants, such as metals and dioxin-like compounds, to WS, or the tolerance of WS in comparison to other fishes. Here, we present the results of studies that investigated effects of selected metals and an arylhydrocarbon receptor (AhR) agonist to ELS WS. WS were exposed to metals in both water and in sediments collected from the upper Columbia River that are hypothesized to be contaminated with metals. In addition, biochemical and molecular effects of the model AhR agonist,  $\beta$ -naphthoflavone ( $\beta$ NF), on juvenile WS were investigated. ELS WS were relatively sensitive to Cd, Cu, Zn, and Pb, but LC values for acute and chronic exposures were greater than the water quality criteria for both the United States and Canada. WS were responsive to AhR agonists and could be among the more sensitive fishes with regard to inducibility of CYP1A. Although the liver is the main location of these responses, other organs such as the gill and intestine have



the capacity to respond as evidenced by both the high level of induction in EROD activity as well as the induction of both CYP1A and AhR transcript abundance. Preliminary results of the sediment exposure study indicate that exposure to contaminants through sediments in the upper Columbia River is unlikely to significantly contribute to the poor recruitment of WS.

**RP230 Assessing environmental parameters and heavy metals in sediment and seabird samples from Greenwich Island, Antarctic Peninsula** P. Calle Delgado, Escuela Superior Politecnica del Litoral / Facultad de Ingeniería Marítima y Ciencias del Mar (FIMCM), Escuela Superior Politecnica del Litoral / Department of Marine Engineering Biological, Ocean Sciences and Natural Resources; L. Monserrate, O. Alvarado, Escuela Superior Politecnica del Litoral / Department of Marine Engineering Biological, Ocean Sciences and Natural Resources; J. Alava, Simon Fraser University / School of Resource and Environmental Management (Faculty of Environment), Fundación Ecuatoriana para el Estudio de Mamíferos Marinos (FEMM) / FEMM Filial Playas, Simon Fraser University / School of Resource and Environmental Management, Faculty of Environment. The Greenwich Island is located at the south of the Shetland Archipelago in the Antarctic Continent. This region has been considered one of the few pristine places of the world; therefore, it is of particular importance to preserve its resources and unique environment. The ongoing global warming the planet is experiencing and the anthropogenic activities occurring all over the world are rapidly affecting this fragile region. Through the scientific expedition program of the Ecuadorian Antarctic Institute, new research is being conducted to assess abiotic variables, biotic components and environmental stressors, including pollutants and climate change, in the Antarctic Peninsula. The aim of this work was to determine: a) the environmental conditions and sediment characterization of the Greenwich Island during the Antarctic summer of 2009, 2011 and 2012; b) levels of copper and mercury in sediment and soil samples of the Greenwich Island; and, c) assessment of mercury accumulation in feathers of Antarctic seabirds, including southern giant petrels (*Macronectes giganteus*), and penguins (*Pygoscelis antarctica*; *P. papua*; *P. adeliae*). To accomplish these objectives, nine sampling sites along the coastal shoreline of Punta Fort Williams (Greenwich Island) and thirty-three sampling locations in the interior were surveyed. Superficial seawater temperature (SST) was found oscillating during the day, ranging from 1.90 °C to 8.90 °C; salinity, from 20.0 ups to 35.0 ups; pH, from 6.80 to 8.70. Lower salinities and acidification conditions (pH < 7) were found closer to fresh water discharges. The sediment composition analysis for the coastal line of Punta Fort Williams showed values (mean ± SD) of 99.0% ± 1.50 of sand, 0.5% ± 1.20 silt; and 0.7% ± 0.45 clay. Sediment analysis shows that the interior of Punta Fort Williams exhibited 91.0% ± 19.0 of sand; 7.2% ± 20.0 silt; and, 1.9% ± 1.00 clay. The increased sea water temperature observed over the years is likely to be a consequence of global warming as current temperatures are greater than those reported in 2006. Assessment of metals concentrations (Hg, Cu) in sediments and seabirds are currently under lab analysis. This is one of the first environmental and eco-toxicological studies in the Antarctic Peninsula and the findings will be used as baseline data and benchmarks, upon which environmental management actions and mitigation strategies are planned to be implemented.

**RP231 Assessing the toxic potency of Aroclor 1268 to piscivorous marine mammals using mink as a mammalian model** W. Folland, Michigan State University; S.J. Bursian, Michigan State University / Department of Animal Science, Michigan State University / Dept. of Animal Science; M.J. Zwiernik, Michigan State University / Department of Animal Science, Michigan State University, Wildlife Toxicology Laboratory / Department of Animal Science, College of Veterinary Medicine. Concentrations of the polychlorinated biphenyl (PCB) mixture Aroclor 1268 are elevated in aquatic biota associated with the LCP Chemicals Superfund site in the Turtle/Brunswick River Estuary (TBRE) in southeastern Georgia. Total PCB concentrations in blubber samples from bottlenose dolphin (*Tursiops truncatus*) within the TBRE have been reported more than 10-fold greater than those observed in adjacent regional estuaries. Presently there is great uncertainty associated with quantifying the toxic potency of exposure to this highly chlorinated PCB mixture. The uncertainty originates from a lack of toxicology data combined with differing characteristics of this mixture as compared to the lesser-chlorinated PCB mixtures, which have been more extensively studied. Aroclor 1268 is extremely hydrophobic and essentially devoid of coplanar PCB congeners that typically drive PCB toxicity through aryl hydrocarbon receptor (AhR) mediated toxic responses. The American

mink is the quintessential mammalian model for understanding mammalian PCB toxicity and is considered to be a suitable surrogate species for cetaceans due to similarities in diet and taxonomic class. Furthermore, mink are known to be exquisitely sensitive to PCBs, thus the application of mink toxicology data to other mammals is expected to provide a level of safety. In an effort to determine toxic reference values for Aroclor 1268 that can be applied to marine mammals and assign a relative potency value for Aroclor 1268 with respect to 3,3',4,4',5-pentachlorobiphenyl (PCB 126), a trial was conducted to assess and compare their effects on reproduction of adult female mink and on survival and growth of their offspring. Dams and kits were fed a ranch diet spiked with 0, 1.7, 4.0, 10, 18, or 29 ppm Aroclor 1268 or 0.0005 ppm PCB 126. Reproductive, hematological, immunological, and histological endpoints were used to derive toxic reference values so that responses to Aroclor 1268 may be more clearly defined.

**RP232 Deriving Generic Soil Preliminary Remediation Goals and Remedial Action Levels for the Protection of Terrestrial Ecological Receptors** L.H. Judd, Environmental Resources Management; S.C. Peterson, Environmental Resources Management (ERM), Environmental Resources Management; K. Fletcher, Environmental Resources Management. With the promulgation of Ecological Soil Screening Levels (Eco-SSLs) by USEPA, procedures for addressing terrestrial ecological risk as a component of State or Federal site remediation projects have become increasingly standardized in recent years. Whereas the Eco-SSLs are a convenient tool for generic use to identify and manage ecological risk issues, according to USEPA, it is inappropriate to use the Eco-SSLs as cleanup standards. However, no acceptable alternatives are offered. In many cases, either for reasons of cost or time, defining appropriate ecological preliminary remediation goals (Eco-PRGs) to address ecological risk and ensure the long term protection of terrestrial ecological receptors must be accomplished in the absence of site-specific information on constituent toxicity or bioavailability. This paper outlines an approach for using the Eco-SSLs to derive generic Eco-PRGs for soil, and further provides an approach to demonstrate compliance with these goals. For avian and mammalian receptors, Eco-SSLs are based on the geometric mean of peer-reviewed no observed adverse effect levels (NOAELs) for growth and reproduction across all test species. Similar to other published reports, generic Eco-PRGs may be based on the geometric mean of the NOAEL and the lowest observed adverse effect level (LOAEL). Important considerations in deriving the Eco-PRGs include the bounding of potential effects between the NOAEL and the LOAEL, and the selection of appropriate effect endpoints and indicator species. Using this methodology, generic Eco-PRGs are tabulated for common constituents in soil. Compliance with generic Eco-PRGs focuses on the derivation of a remedial action level (RAL), which is defined as the maximum concentration that can be left in place within a study area that would result in average exposure to soils (estimated by the arithmetic average or an upper confidence limit on the mean) equal to or less than the generic Eco-PRG. An example of the application of this methodology is provided.

**RP233 Development of Fish Tissue Screening Values for the Protection of Piscivorous Ecological Receptors** M. Bowersox, Tetra Tech, Inc. / Laboratory Department; P. Siwinski, J. Diamond, Tetra Tech, Inc.; F. John, L. Hunt, US EPA – Region 6. The potential ecological effect of contaminants of concern in fish tissue is not easily determined due to the lack of fish tissue screening values for the protection of piscivorous fish and wildlife. USEPA was interested in determining potential ecological risks to fish and higher trophic levels based on fish tissue concentrations measured in their National Coastal Condition Assessment. Contaminants of concern included pesticides, metals, PCBs, dioxins, and PAHs, all of which lacked fish tissue screening values for ingestion by piscivorous birds and wildlife. In this study, exposure concentrations for piscivorous receptors were based on organism body weight, food ingestion rate, habitat use factor, and concentration in diet (fish tissue concentration). Effect concentrations for contaminants were derived from laboratory exposures reported in scientific literature. By setting the risk equal to 1.0 and applying the known effect level, as well as body weight and food ingestion rate, an upper bound “safe” fish tissue concentration was calculated and used as a screening value for each contaminant. Receptors were categorized as either birds, freshwater fish, marine fish, freshwater mammals, and marine mammals. Exposure variables, including body weight and food ingestion rate, were evaluated for specific receptors within each category and a general body weight and food ingestion rate were used for each category based on the most sensitive case

(i.e., minimum body weight and maximum food ingestion rate). Fish tissue screening values were developed for each category of receptor and each contaminant of interest. The minimum screening value for each contaminant was identified and should be protective of all piscivorous ecological receptors. Further requirements being considered include evaluation of specific receptors in certain coastal regions of the U.S. and use of tissue screening values to identify potential areas of concern and high priority contaminants for further evaluation.

**RP234 Inclusion of soil arsenic bioaccessibility in ecological risk assessment and comparison with biological effects** J. Saunders, Stantec Consulting Limited; L. Knopper, Intrinsik Environmental Sciences Inc; I. Koch, Royal Military College of Canada; K. Reimer, Royal Military College of Canada. The purpose of this study was to conduct an ecological risk assessment (ERA) for meadow voles (*Microtus pennsylvanicus*) found at three arsenic contaminated sites in Nova Scotia, Canada (as well as two background locations) and to compare the numeric results to measured biomarkers of exposure and effect. The daily intake of arsenic by meadow voles was determined by three separate calculations: estimated daily intake (EDI), bioaccessible estimated daily intake (BEDI, with bioaccessibility of soil included), and actual daily intake (ADI, which is calculated with arsenic concentrations in the stomach contents). The median bioaccessibility of arsenic in soils from the contaminated locations was significantly greater than at background locations. The bioaccessible arsenic concentration in soil from all samples (both contaminated and background) was significantly less than the total concentration. Use of site-specific bioaccessibility (hazard quotients=38 at Upper Seal Harbour (USH); 60 at Lower Seal Harbour (LSH); and 120 at Montague tailings (MONT)) and stomach arsenic contents (hazard quotients=2.1 at USH; 7.9 at LSH; and 6.7 at MONT) in the ERA resulted in lower numeric risk than compared to risk calculated with 100% bioavailability (hazard quotient=180 at USH; 75 at LSH; and 680 at MONT). Further, the use of bioaccessibility on the calculation of risk was aligned with biomarker results (changes in glutathione and micro-nucleated erythrocytes) in voles captured at the sites. This study provides evidence that using site-specific bioaccessibility in ERAs may provide a more realistic level of conservatism, thereby enhancing the accuracy of predicting risk to wildlife receptors. Furthermore, when numeric risk assessments are combined with site-specific biological data (i.e., biomarkers of exposure and effect), both lines of evidence can be used to make informed decisions about ecological risk and site management.

**RP235 Physiological Effects of Ingestion of Latex Balloon Fragments on Wildlife** S.W. Irwin, Clemson University / School of Agriculture, Forestry, and Environmental Sciences; W. Bowerman, Clemson University / Forestry & Natural Resources; W. Bridges, Clemson University / Department of Mathematical Sciences; H. Irwin, Clemson University. For many years mass latex balloon releases have been used to celebrate a variety of public events from holidays to sporting events. Concern has risen over these releases as to what risk the balloons pose to wildlife. This concern is based on the detrimental effects on the environment and wildlife from plastic waste and litter, although natural latex balloons are not plastic. This study examined the potential threat that latex balloon fragments may represent to wildlife through ingestion. Trial species of Japanese Quail (*Coturnix coturnix japonica*), Red-eared Sliders (*Trachemys scripta elegans*) and Channel Catfish (*Ictalurus punctatus*) were model species representing wildlife taxa potentially impacted by latex balloons. Latex fragments were offered for consumption for four weeks. Blood samples were taken pre- and post-trial to discern any change in heterophil to lymphocyte (H/L) or neutrophil to lymphocyte (N/L) ratios as an indicator of physiological stress from ingestion. Weight was recorded weekly. Test organisms were euthanized at four weeks and necropsies were performed to examine for digestive tract anomalies. No significant difference was detected in H/L ratios pre- and post-trial for *C. coturnix japonica* ( $m = 0.45$  and  $0.39$ , respectively;  $P = 0.11$ ) or for *T. scripta elegans* ( $m = 0.39$  and  $0.45$ , respectively;  $P = 0.24$ ). There was a significant decrease in N/L ratios from pre- to post-trial for *I. punctatus* ( $m = 0.04$  and  $0.02$ , respectively;  $P = 0.02$ ). Weight increased significantly for sub-adult quail and catfish fingerlings during the study ( $P < 0.0001$ ); no significant change of weight was observed in adult turtles. Necropsies did not reveal any digestive anomalies in quail or catfish; although turtles did show substantial accumulation of latex fragments in three of 14 specimens (21%). Results of this study suggest that consumption of latex balloon fragments may not pose

a threat to many wildlife species. However, further long-term studies on turtles may be warranted.

**RP236 Restoring Aquatic Connectivity and Fish Passage in Menominee River, WI: An Assessment of the Potential Risks from Contaminants** S. Warner, U.S. Fish and Wildlife Service / Environmental Contaminants Program. The removal of dams and the creation of fish passage bypass in dams are projects designed to restore aquatic connectivity. Problems with contaminants can occur if fish passage projects take place in polluted ecosystems. This poster explains the environmental risks associated with fish passage projects. A fish passage case study for a Wisconsin river is presented describing the potential contaminant risks of downstream and upstream fish passage. A baseline risk assessment that numerically estimates risk to upstream wildlife from passing downstream lake sturgeon (*Acipenser fulvescens*) contaminated with polychlorinated biphenyls (PCBs) is explained. The bald eagle (*Haliaeetus leucocephalus*) is used as a bioindicator species for piscivorous wildlife in the baseline risk assessment. The assessment determined that although risk does exist, it is likely below the level that adversely affects bald eagle reproductive success. The assumptions and uncertainties of the risk assessment and additional considerations for determining risk are discussed. Conducting baseline risk assessments and monitoring contaminants in wildlife should be used to determine potential environmental impacts of fish passage projects.

**RP237 Implications in Using Bioaccumulation Models in the Development of Soil Risk-Based Screening Levels Within Ecological Risk Assessments** K.M. Rettmann, V.C. Chen, B.A. Narloch, MWH Americas, Inc.. The development of chemical-specific, ecological risk-based screening levels (EcoRBSLs) are often prepared for purposes of site characterization, in ecological risk assessments, and for aiding in risk management decisions regarding site cleanups. Chemical-specific, soil EcoRBSLs developed for upper trophic level receptors such as mammals and birds are receptor-specific models that account for the life history of each representative receptor, toxicity reference values, and bioaccumulation models (empirical uptake factors or regression models). Bioaccumulation regression models can either be based on simple lognormal-to-lognormal or log-to-log regression models or based upon the upper 95% prediction limit for a simple lognormal-to-lognormal or log-to-log regression models. In studies where bioaccumulation models are compiled or developed, regression models are often recommended for use over simple uptake factors, because regressions tend to provide better estimates of concentrations of chemicals in biota or prey items. EcoRBSLs can be calculated for chemicals with bioaccumulation regression models by using the Solver analysis tool in Excel. However, when comparing ecological hazard quotients (HQs) estimated using a standard forward risk calculation to HQs estimated using the ratio of an exposure point concentration (EPC) to an EcoRBSL calculated using bioaccumulation regression models, the difference in HQs may range up to several orders of magnitude, depending upon the EPC. For example, when the EPC is at or around the value of the estimated EcoRBSL, there is little to no difference in HQs estimated using the two approaches. As the difference between the EPC and the EcoRBSL increases, the difference between the two types of HQs tends to increase. These results suggest that, while bioaccumulation regression models tend to provide better estimates of concentrations of chemicals in biota and prey items than do bioaccumulation factors, the use of bioaccumulation regression models to derive EcoRBSLs should be used with caution.

**RP238 An Analysis of the Risks and Benefits of Fish Consumption in the Pacific Northwest** J. Stiles, Western Washington University / Huxley College of the Environment; M. Ponce-McDermott, D. Wood, Western Washington University; R.M. Sofield, Western Washington University / Huxley College of Environment. We examined the risks and benefits of fish consumption from the Puget Sound in light of the strong cultural traditions of the PNW Native American tribes. The Puget Sound has been found to have relatively high levels of PCBs and PAHs, causing the 2007 Washington State legislature to fund a Puget Sound initiative that includes support for the Department of Ecology to cleanup the majority of the sites in the Puget Sound by 2020. Despite high chemical concentrations, cultural traditions of fish consumption are strong, but are being limited by the potential risks of consuming the contaminated fish. This study analyzed much of the current research behind this question of risks and benefits keeping the cultural importance of fish to Native American peoples at the forefront. Results of this literature review and analysis will be presented.

**RP239 Assessment of Ecological Risks to a Marine Benthic Fish and a Marine Piscivorous Bird at Saglek Bay, a PCB Contaminated Site in Northern Labrador** K.E. Johnson, Stantec Consulting; C. Ollson, Intrinsiq; D. Schneider, Memorial University of Newfoundland; K. Reimer, Royal Military College. An ecological risk assessment was conducted for short-horn sculpin (*Myoxocephalus scorpius*) and black guillemot (*Cepphus grylle*) living near a polychlorinated biphenyl (PCB) contaminated beach at Saglek, Labrador. The ERA was conducted based on information collected from two time periods (1998/1999 and 2006/2007) during which total PCBs in sediment and biota tissues were observed to decrease substantially. A screening level hazard quotient approach was initially applied. Site-specific biological effects including biomarkers and condition indices were further examined in a weight of evidence approach to evaluate the effectiveness of the lower tiered assessment. Hazard quotients from both time periods indicated that black guillemots could be experiencing adverse health effects as a result of their exposure to PCBs. This was supported by the biomarker based weight of evidence approach which indicated an intermediate to high risk to black guillemots. Hazard quotients for the shorthorn sculpin on the other hand indicated potentially adverse health effects in 1998/1999 but not in 2006/2007. The weight of evidence supported the hazard quotients indicating an intermediate risk in 1998/1999 and a low risk in 2006/2007. Despite the predicted health effects to black guillemots at Saglek, the population appears to be thriving. Measurement of population indices would be required to confirm the predicted adverse effects on black guillemots. A three tier iterative approach utilizing hazard quotients, biomarkers, and population and/or community studies is recommended for large complex sites such as Saglek, where remediation strategies are expensive and potentially destructive to the environment. This study emphasises field verification of adverse health effects predicted through the screening (*i.e.*, hazard quotient) assessment stage and supports an iterative tiered approach to ecological risk assessment.

**RP240 Polychlorinated Naphthalene, Another PCBs?** C. Julias, CDM, CDM Smith / Chemical Engineer, CDM / Chemical Engineer; T. Marcum, CDM Smith / Environmental Scientist; N. Luke, CDM, CDM Smith Inc.. Polychlorinated naphthalenes (PCNs) are a group of substances based on the naphthalene ring system. They are a group of 75 compounds consisting of two fused aromatic six member rings substituted with one to eight chlorine atoms. They are structurally similar to the polychlorinated biphenyls (PCBs). However, unlike PCBs, little information exists on PCNs. PCNs were first synthesized in the middle of the 19th century and subsequently were found to possess good electrical insulation properties, excellent weather resistance, and low flammability. Production of PCNs in the United States and Europe ceased in the 1980s. Current emissions of PCNs are caused by unintentional releases from combustion processes to produce heat and power, from industrial processes, solvent use, and waste incineration. Similar to PCBs, PCNs are persistent and lipophilic compounds that tend to bioaccumulate. Concerns and risks related to its production and use have allowed PCNs to be considered a persistent organic pollutant (POP). POPs are extremely toxic substances for environment and human health at a world scale. Their physical and chemical properties, particularly their high stability, give them ubiquity and capacity of accumulation in the leaving organisms and nature. Insufficient evidence is available on persistence of PCNs in the environment. However, recent work has been done to determine the relative potency of PCNs – mixtures as well as individual congeners – in fish, birds, and mammals. The potency of several PCN congeners is in the same range as some PCB congeners. In addition, the pattern of toxicity of PCNs resembles that of tetrachlorodibenzodioxin. The relative order of potency, along with the wide distribution of PCN contamination in the environment, suggests that monitoring programs should be extended to include this persistent substance.

**RP241 Water quality and fish edibility: A human health risk assessment** G. Wagenaar, A. Mooney, University of Johannesburg / Department of Zoology; C. van Dyk, University of Johannesburg / Department of Zoology, Centre for Aquatic Research. Bioaccumulation of pollutants in fish tissues could compromise the edibility of the fish, making them potentially unsafe for human consumption. The aim of this study was to determine whether the freshwater fish species *Oreochromis mossambicus*, from three impoundments known to be polluted by extensive mining and agricultural activities, were safe for human consumption. The objectives were: (1) to conduct chemical water analyses to identify levels of inorganic and organic chemical

contamination; (2) to do a standard fish necropsy and collect tissue samples for a histology-based fish health assessment on selected target organs (gills, liver, kidney, heart, testes and ovaries); (3) to analyse fish muscle samples for levels of inorganic and organic pollutants and; (4) to undertake a human health risk assessment regarding the edibility of the fish, to determine the potential toxic risk of all chemicals present, as well as the cancer risk of the carcinogens identified to be present in the muscle. The results from the chemical water quality analyses indicated that concentrations of various organochloride pesticide namely; Aldrin, *p,p'*-Dichlorodiphenyltrichloroethane (DDT) and *p,p'*-Dichlorodiphenyldichloroethane (DDD), were found to be above the acceptable ranges. The necropsy observations showed no macroscopic abnormalities. The qualitative histological analysis showed alterations in all the target organs besides the testes. The most affected organs in terms of the number of histological alterations identified were the livers and kidneys. Despite the histological alterations identified, the organs still seem to be functioning normally, which is likely to be due to regulatory mechanisms of the fish. Results from the edibility studies and the associated human health risk assessments, showed that none of the bio-accumulated pollutants were posing any toxic risks, although, cancer risks were identified for arsenic and DDE, but these were not unacceptably high. It was concluded that the water quality in all the impoundments, including the reference site, has been compromised by anthropogenic activities. In terms of edibility it was identified that even at worst case scenario, no considerable human health risks were posed by fish consumption from any of the impoundments. It is therefore likely that currently the nutritional benefits of fish consumption still outweigh the risks.

**RP242 A Re-evaluation of Great Lakes Fish Passage and Effects on Bald Eagles Nesting Along the Au Sable, Manistee and Muskegon Rivers** P. Datema, Clemson University / Wildlife and Fisheries; W. Bowerman, University of Maryland / Department of Environmental Science & Technology; T. Grubb, United States Forest Service; W. Bridges, Clemson University. Consumers Energy operated hydroelectric projects located along the Au Sable, Manistee, and Muskegon Rivers underwent environmental studies in the late 1980s and early 1990s as part of the Federal Energy Regulatory Commission relicensing. It was questioned if passage of Great Lakes' fishes over barrier dams along these rivers would cause detrimental impacts to sensitive wildlife species. Bald eagles were chosen as a biomonitor. This risk assessment included calculating new hazard quotients (HQs) from toxic reference values (TRVs) to determine if it was safe for inland wildlife to be exposed to anadromous fish allowed past barrier dams. A risk assessment was conducted for contaminants of PCBs, DDT, dieldrin, TCDD-EQ and mercury in a fish diet comparing exposure in Great Lakes' accessible regions to interior regions of the Au Sable, Manistee and Muskegon rivers, using fish collected after 1990. The bald eagle population nesting in the study area increased throughout the study period. Mean total PCBs, sum DDT and dieldrin were greater in Great Lakes influenced areas. Total PCBs and sum DDT were greater in Great Lakes influenced nesting areas than inland nesting areas. TCDD-EQ was the limiting factor for bald eagle reproduction on Great Lakes influenced areas with the greatest HQ, which was greater than the adverse population level. My data suggests that if protection of wildlife from environmental contaminants is the management goal, then fish passage should not be allowed past Foote, Tippy and Croton dams. Concentrations of environmental contaminants in nestling bald eagle blood plasma confirm these results.

**RP243 Community-wide measures of streams as ecological indicators to assess the effects of land cover change at basin scale** L. Carrasco-Letelier, National Agriculture Research Institute / National Research Program for Production and Environmental Sustainability, National Agricultural Research Institute (INIA Uruguay) / Production and Environmental Sustainability; C. Suarez-Pirez, Faculty of Sciences, University of Republic; G. Eguren-Iriarte, Universidad de la República / Maestría en Ciencias Ambientales; K.R. Munkittrick, University of New Brunswick / Canadian Rivers Institute, University of New Brunswick / Department of Biology. Standards of freshwater quality for watershed land cover change have yet to be developed for Uruguayan, and countries of South American basins at Atlantic neotropic ecozone. In this framework, efforts have been made toward to characterizing the changes of ecosystem's trophic structure. However, in important land cover changes, as the Uruguayan Eucalyptus plantations in grassland ecosystems; with biogeochemical changes in soils, at plot level, and in freshwater quality, at basin scale, are not clear defined by classic ecology



parameters (diversity, evenness). This situation limits the interpretation of the ecological consequences of the land cover change at basin scale. Information that is necessary both to prevent not desirable problems of ecosystem and agriculture production, and to define standard environmental quality indicator for watershed. In Uruguay, the fast growing forest plantations increased, in the last 20 years, its land use to approximately 900,000 ha. The present work carried out a biological comparison of the river's ecosystem in two representative basins with different land cover use (Eucalyptus plantation vs traditional agriculture). Determination complemented with a stable isotope analysis of  $^{13}\text{C}$  and  $^{15}\text{N}$ . The results shown: three common fishes species in a total of 23 species, none difference in diversity and evenness; but changes in  $^{13}\text{C}$  and  $^{15}\text{N}$  ratio of fish communities. These results allow to propose the Layman's community-wide measures (NR, CR, TA, CD, NND, SDNND) as potential indicators for the ecological risk assessment of basin's land use change.

**RP244 Finding, Mapping, and Measuring Oil in the Ocean Using Aerial Remote Sensing** J. Svejksky, Ocean Imaging Corp.; J. Mullin, Joseph Mullin Consulting, LLC; B.M. Joab, CA Dept. of Fish and Game, Office of Spill Prevention and Response / Office of Spill Prevention and Response; J. Muskat, California Department of Fish and Game / Office of Spill Prevention and Response. Rapid access to data and information by different pollution response groups is vital during oil spill response activities in order to minimize exposure and risk to wildlife and aquatic organisms. While some information can be relayed verbally, other data such as the exact extents of the spill and oil thickness distributions within it are best disseminated in the form of graphics. Ocean Imaging Corp., with support from California's Department of Fish and Game Office of Spill Prevention and Response (CDFG-OSPR) and the U.S. Bureau of Safety and Environmental Enforcement (BSEE, formerly the US Mineral Management Service and Bureau of Ocean Energy Management, Regulation, and Enforcement), have developed a versatile aerial remote sensing package and a companion web-based Geographical Information System (GIS) specifically for oil spill response that enables rapid access to various types of data by office and field personnel. The system is based around a multispectral camera and a thermal imager integrated with a GPS based inertial measurement unit positioner which is used to image the oil spill region in 4 visible and 1 thermal IR wavelength channels. A newly developed, neural network-based oil film thickness algorithm is then applied to the image data in near-real-time to create a digital oil distribution class map product. The map can be uploaded onto the GIS server directly from the aircraft and immediately utilized to coordinate and guide pollution response activities. Using funding from OSPR's Scientific Study and Evaluation Program (SSEP) and from BSEE this system was developed and later applied on a test basis during the M/V Cosco Busan spill in San Francisco in 2007. Subsequently it has been utilized operationally for other oil spills including the Deepwater Horizon event in the Gulf of Mexico in 2010. After the Deepwater Horizon experience the companion GIS was dropped in favor of the National Oceanic and Atmospheric Administration's (NOAA's) Environmental Response Management Application (ERMA), a web based GIS designed to be a new national standard for oil spill response. Application of this technology allows for more efficient utilization of oil spill cleanup resources, and ultimately may result in reduced exposures of wildlife and aquatic organisms to spilled oil.

**RP245 Reactivation Furnace Screening Level Ecological Risk Assessment Case Study for Calgon Carbon Big Sandy Plant** M. Weisberg, J. Brooker, Shaw Environmental, Inc. / Dept. of Risk Assessment. A screening level ecological risk assessment (SLERA) evaluated potential risks from emissions from the carbon reactivation furnace located at the Calgon Carbon Big Sandy Plant in Kentucky. Emission sources and rates were identified through the performance of a Risk Burn, and chemical results for 270 chemical constituents were used. Air dispersion modeling was used to estimate potential concentrations and deposition rates of emitted constituents at various receptor locations within a 50-km modeling domain. This information was then used to determine potential exposure and assess bioaccumulation. Equations were used to estimate constituent concentrations in soil, surface water, and sediment and a variety of biological receptors. Within the 50-km modeling domain the maximum exposed individual (MEI) terrestrial receptor location was situated 1,400 feet E-NE of the Plant across the Big Sandy River in deciduous forest. A more detailed evaluation was performed for the dozens of waterbodies and river segments to identify critical waterbodies expected to be most impacted. To identify

critical waterbodies, key characteristics of each were tabulated, including those factors expected to most influence constituent concentrations in fish, surface water, and sediment. Based on this analysis, five critical waterbodies were selected for quantification in the SLERA. The three food webs investigated included (1) forest (terrestrial hardwoods); (2) scrub/shrub (terrestrial freshwater wetlands); and (3) freshwater (aquatic) ecosystems. Fifteen wildlife receptors were quantitatively evaluated. Also evaluated were soil, surface water, and sediment communities potentially exposed to constituents in these media. Ecological effects quotients (EEQ) were calculated for each constituent for each wildlife receptor at the MEI terrestrial and five aquatic exposure scenario locations. Estimated constituent concentrations in abiotic media were compared with direct contact toxicity reference values to assess potential impacts on ecological communities. Based on a detailed evaluation of the EEQ constituent risk drivers and pathways, none of the 15 wildlife species had EEQs above 1. Similarly, no estimated constituent concentrations in soil, surface water, or sediment exceeded direct contact toxicity reference values; therefore, potential impacts on ecological communities associated with these media were not a concern.

**RP246 Using a Bayesian network approach to evaluate the effectiveness of low impact development techniques in managing risk in the Puyallup River Watershed** S. Edwards, Western Washington University / Institute of Environmental Toxicology; E.E. Hines, Western Washington University / Institute of Environmental Toxicology, Huxley College of the Environment; W.G. Landis, Western Washington University, Institute of Environmental Toxicology / Western Washington University, Western Washington University / Institute of Environmental Toxicology, Western Washington University / Institute of Environmental Tox. & Chem.. Regional risk assessment is a framework used to calculate the likelihood of specified impacts to a set of identified endpoints in complex, ecological systems where multiple stressors are present. The area of interest is broken into risk regions based on habitat and land use. We will be evaluating the risk associated with various natural and anthropogenic stressors to stakeholder endpoints in the Puyallup River watershed, as well as the effectiveness of various low impact development practices in reducing risk to the endpoints. There are five risk regions: the eastern and western portions of the White River – Lake Tapps River basin, the Carbon River – Upper Puyallup River basin, the Mid-Puyallup River basin and the combined area of the cities of Tacoma, Federal Way, Fife and the Clark's Creek River basin. Bayesian networks (BNs) are a tool used to formulate the relative risk rankings for each region. The structure of BNs consists of the following three tiers of nodes: landscape stressors, habitats, and ecological endpoints of value to stakeholders. The nodes of each tier link are linked to lower nodes if ecological or spatial relationships exist. For each parameter, four potential discrete states exist: zero, low, medium, or high with the assigned numerical score of 0, 2, 4, and 6 respectively. Management strategies, including the implementation of low impact development practices, will be evaluated for effectiveness in reducing risk to the endpoints. Associated uncertainty will be explicitly stated. Management goals are derived from the Puget Sound Partnership's goals for restoration of the Puget Sound.

**RP247 Environmental risk assessment for shipwrecks: a fault-tree model to assess probability of contaminant release** H. Landquist, I. Hasselov, Chalmers University of Technology / Shipping and Marine Technology; L. Rosen, Chalmers University of Technology / Civil and Environmental Engineering; F. Lindgren, Chalmers University of Technology / Shipping and Marine Technology; I. Dahllof, The University of Gothenburg; T. Norberg, Chalmers University of Technology / Mathematical Sciences; A. Lindhe, Chalmers University of Technology / Civil and Environmental Engineering. Shipwrecks lying on the sea floor around the world may contain hazardous substances which can cause harm to the maritime environment. Today there are no comprehensive methods for environmental risk assessment of shipwrecks and thus there is poor support for decision-making on prioritisation of mitigation measures. The purpose of this study has been to develop a framework for risk assessment of potentially polluting shipwrecks, focusing on a detailed probabilistic assessment of the release of hazardous substances. The framework is based on international standards for risk management and the assessment of the probability for release was done using fault-tree analysis. This approach enables a structure assessment and provides an excellent possibility of uncertainty and sensitivity analysis. The fault tree model was tested by groups of experts in the field of maritime administration, maritime archaeology and in other areas to validate the method. The developed

framework facilitates risk assessment of potentially polluting shipwrecks and can support risk management as well as provide well founded input for decision-making. In particular the fault-tree analysis provides a quantitative method for assessing the probability of leakage and can facilitate uncertainty and sensitivity analyses.

**RP248 Assessment of Bioaccumulation and Ecological Risk for an Intertidal Reef Flat Environment at Wake Atoll** D.W. Shelton, J.M. Cotter, J.D. Schut, R.D. Ketz, CH2M Hill; S.M. Hunt, U.S. Air Force 611th Civil Engineer Squadron. The potential for bioaccumulation and ecological exposure was evaluated in support of a Baseline Ecological Risk Assessment at a metal debris area located within an intertidal reef flat environment at the southern tip of Wake Island, part of Wake Atoll. Wake Atoll is an unincorporated territory of the United States located about 2,400 miles west of Honolulu, Hawaii, and is a component of the Pacific Remote Islands Marine National Monument and Wake Atoll National Wildlife Refuge. The marine habitats offshore of the site consist of fringing coral reefs, shallow reefs, patch corals, tidal pools, and sand beaches. A wide variety of fish, coral, and sediment-dwelling invertebrate species are found in the marine habitats. The risk assessment investigated whether the debris had affected sediment at levels believed to pose unacceptable risks to marine organisms living on the intertidal reef flat. It was also suspected that the contaminants in the sediment and metal debris were bioaccumulating in fish, which may pose unacceptable risks to seabirds and other consumers of fish. Multiple lines of evidence were assessed to support a conclusion regarding potential impacts from the debris. These line of evidence included chemical analysis of sediment samples, measurement of accumulation of site-related chemicals into predatory fish, sediment toxicity testing, and an intertidal community abundance and diversity survey. The samples were collected within and in the immediate vicinity of the site, as well as from a designated Reference Area located in a similar environment. The peppered moray eel (*Gymnothorax picta*; syn. *Siderea picta*), was selected as a representative species of marine aquatic receptors that might bioaccumulate contaminants released at the debris site. This eel is widespread in the tropical Indo-Pacific region, occurring strictly in intertidal areas of reef-associated coralline and rocky bottom marine habitat. They are considered territorial top-end carnivores. Results of the risk assessment confirmed that marine sediment and biota were being affected and the source of contamination was identified as the mass of metal debris at the site. These results have supported a decision to restore the intertidal area.

**RP249 Assessment of herbicide effects on the clitellata and ecological function in paddy field** S. Yachi, National Institute for Agro-Environmental Sciences / Researcher; N. Kaneko, Yokohama national university. Sediments provide a habitat for various benthic animals that have an important contribution to functioning of aquatic ecosystems. In addition, animals in sediment are exposed to chemical substances longer than those in the overlying water because of their residue. This study assessed the effect of herbicide on clitellates in paddy and estimated the loss of ecosystem function sustained by clitellata. Field surveys of clitellate were conducted between June 2010 and May 2011 and the community was dominated by *Limnodrilus hoffmeisteri* and *Branchiura sowerbyi*. In May, *B. sowerbyi* had high number of reproducing individuals (1809 ind. m<sup>-2</sup>). Toxicity test on PCP (Pentachlorophenol) and pyrazolate were conducted for these clitellata species and a standard test species (*Lumbriculus variegatus*). PCP is the most popular herbicide in Japan in the 1960s and the 1970s and it has high toxicity. Pyrazolate is currently used in Japan and usually sprayed in May. The herbicidal effect is obtained by the slowly hydrolysis in the soil over a long duration. Regular dose of PCP and pyrazolate were 140 mg/kg soil and 15 mg/kg soil, respectively. In the case of PCP, 28 days LC50 (mortality) values for *B. sowerbyi* was 119.8 mg/kg. Meanwhile, pyrazolate has low mortality effect (>1000 mg/kg) but EC50 (reproduction) was 30.1 mg/kg. The role of clitellata as ecosystem functioning revealed by laboratory incubation is that they translocated lower sediment soil to surface, and increased NH<sub>4</sub><sup>+</sup> concentration of over laying water and PO<sub>4</sub><sup>-</sup> concentration of soil in density dependent manner. Therefore we estimated that during the period of applying PCP, the ecosystem functioning by clitellate has been lost at least 50% for soil translocation and nutrient cycling. Pyrazolate may decrease population of *B. sowerbyi* in long time, because application time coincides with reproduction season. It may cause decreasing ecosystem functioning as a result of used this chemical.

**RP250 Comparison of One-Carbon and Two-Carbon Equilibrium Partitioning Modeling for PAHs in Sediment** R. Heslep, ARCADIS / Risk Assessment and Ecological Services; C. Day, S. Sullivan, ARCADIS. The standard approach described by the United States Environmental Protection Agency (EPA) for predicting pore-water concentrations of polycyclic aromatic hydrocarbons (PAHs) in sediment utilizes a one-carbon equilibrium partitioning model that incorporates total organic carbon (TOC) and the measured bulk sediment concentration of individual PAHs. However, the EPA has recognized that various types of carbon have differing capacity for binding PAHs. One such type of carbon that has been shown to have a significant binding capacity is black carbon (anthropogenic carbon). In addition to natural organic matter such as vegetative debris, humic and fulvic acids, and decayed remains of plants and animals which all contain non-pyrogenic organic carbon, sediment organic carbon is also comprised of black carbon particles such as coke, charcoal, and soot, which are known to have extremely high sorption capacities. Impacted sediments from industrial sites can contain particularly high levels of anthropogenic black carbon. Whereas the one-carbon equilibrium partitioning model is still widely used in developing sediment quality guidelines for PAHs based on final chronic values of PAHs in the equilibrium water phase, the presence of black carbon in sediments may make this approach overly conservative. The partitioning of each PAH through organic carbon absorption and adsorption onto black carbon allows the estimation of the dissolved concentration of each PAH in sediment pore-water. A comparison of the Toxic Unit (TU) results using the one-carbon approach (based on TOC for natural organic carbon) and two-carbon approach (based on the sorption to black carbon and to TOC) will be presented for sediment samples collected from a freshwater marsh. The influence of black carbon on the bioavailability and estimated toxicity of PAHs to benthic life will be presented.

**RP251 Mercury Investigation at Site 1 – Dodge Pond, East Lyme, Connecticut** A. Bernhardt, Tetra Tech; D. O'Connor, V. Jurka, J. Wright, Naval Facilities Engineering Command, Mid-Atlantic; M. Hammond, Naval Undersea Warfare Center; L. Ganser, Tetra Tech. Dodge Pond is a 33-acre kettle lake, located in East Lyme, Connecticut, used by the Navy for acoustic testing and evaluation. The land surrounding Dodge Pond is mostly residential, with forested, undeveloped land to the northwest and an industrial area to the southeast. There is a mercury fish advisory in effect for Dodge Pond due to high mercury concentrations in fish. Mercury was previously detected at elevated levels in sediment and fish tissue, but no primary source of mercury to Dodge Pond has been identified. It is likely that multiple small sources resulted in the elevated levels of mercury in various media in Dodge Pond. The Navy collected sediment, fish tissue, and bird guano samples to obtain current data and to determine the lateral and vertical extent and historical trends of mercury concentrations in sediment. Specifically, the data were collected to determine: 1) whether mercury concentrations in sediment and fish have decreased from previous levels, 2) the lateral and vertical extent of mercury concentrations in sediment in selected portions of the pond, 3) whether two on-shore areas are potential current or historical sources of mercury to Dodge Pond, 4) whether mercury in bird guano may be a current source of mercury to the pond, 5) whether the fish consumption advisories for humans are still appropriate, and, 6) whether there are impacts to piscivorous wildlife from consumption of fish. The investigation found that mercury concentrations in sediment and fish tissue have decreased since the last sampling event (approximately 70 to 90 percent in sediment, and 25 percent in fish tissue). The data do not indicate a continuing source of mercury to the sediment or the source has remained constant in recent years and it does not appear that the two potential on-shore sources contributed mercury to the pond. The current fish advisory in place at Dodge Pond is the more stringent fish consumption advisory the State uses when the arithmetic mean of mercury concentration in fish is 1 milligram per kilogram (mg/kg) or greater. Because the mean mercury concentration in fillet fish tissue samples is less than 1 mg/kg, the less stringent fish consumption advisory may be appropriate. Finally, potential risks to wildlife from consumption of fish from Dodge Pond are expected to be low and bird guano is not likely a current significant source of mercury to Dodge Pond.

**RP252 Sediment resuspension affects metal bioavailability** K.J. Fetters, University of Michigan / School of Natural Resources and Environment; A. Burton, University of Michigan / School of Natural Resources & Environment and Cooperative Institute for Limnology & Ecosystem Research; D.

Costello, A. Harrison, M. Eggleston, University of Michigan / School of Natural Resources & Environment. Sediments in navigation-dominated waterways are frequently contaminated with a wide range of chemicals and are subject to repeated resuspension events. There is little information documenting whether adverse ecological effects result from these resuspensions of contaminated sediments. We studied the release of metals during repeated resuspensions of marine and freshwater sediments and whether or not this resulted in toxicity to organisms. Sediment resuspension events were simulated using Gust Erosion Microcosm Systems (U-GEMS) and Sediment Flux Exposure Chambers (SeFEC) to resuspend metal-contaminated sediments from Lake DePue (IL), San Diego Bay, and Portsmouth Harbor (NH). Various resuspension events of relevant total suspended solid concentrations (200-1000mg/L) were performed. Short-term resuspension events (4 hr and 1 hr on 4 occasions) in the SeFEC resulted in a small percentage of total metals being released into the dissolved phase. Repeated resuspensions showed both release and sorption of metals, which suggests changes in metal partitioning during resuspension. Toxic effects on organisms were limited (*Hyalella azteca*, *Daphnia magna*, and *Neanthes arenaceodentata* survival, QwikLite bioluminescence) over both the time period of the exposure and during a 10 d post-recovery period. To further assess metal release and toxic effects from resuspension, longer-term experiments under static conditions, were completed in the U-GEMS. Baseline conditions (no resuspension) and periodic resuspension events were simulated over 14 d. Periodic resuspension resulted in lower overall concentrations of dissolved Zn compared to the baseline, but also decreased survival for caged *H. azteca* (baseline:  $55 \pm 21\%$ , resuspension:  $10 \pm 14\%$ ). Corresponding *H. azteca* tissue Zn concentrations were elevated due to periodic resuspension (baseline:  $252 \pm 46 \mu\text{g/g dw}$ , resuspension:  $930 \mu\text{g/g dw}$ ). We conclude that sediment resuspension events at relevant TSS concentrations do not release large amounts of dissolved metals, most likely due to the oxidation of reduced iron and sulfide species and subsequent reabsorption by iron oxides. While the release of dissolved metals are low, resuspensions events still pose the risk of causing toxic responses in static environments of low water exchange due to remobilization of contaminated sediments.

**RP253 Use of lead isotopes to identify sources and routes of pollution in Mexican mining sites** R.Y. Perez Rodriguez, Universidad Autónoma de San Luis Potosí, Programa Multidisciplinario de Posgrado en Ciencias Ambientales / Instituto de Geología, Laboratorio de Geoquímica; G. Castro Larragoitia, Universidad Autónoma de San Luis Potosí (UASLP) / Facultad de Ingeniería; F. Diaz-Barriga Martinez, Universidad Autónoma de San Luis Potosí (UASLP) / Facultad de Medicina; M. Alfaro De la Torre, Universidad Autónoma de San Luis Potosí (UASLP) / Facultad de Ciencias Químicas. Mining and refining activities can pollute soils, sediments and waters through the dispersion of tailings and smelter residues and emissions containing metals and other elements. This is the case of the mining district of Villa de la Paz-Matchuala, where lead pollution has been previously reported in soils, sediments and groundwater. Health affections in the local community have also been reported, but it has not been proved that the origin of blood lead concentrations of children living near the tailings is a consequence of mining activities. Another site is Cedral, a district in which the extracted ore from surrounding mines was processed, leaving the residues in open areas. Both sites are located in San Luis Potosí in the northern part of Mexico. To trace the origin of lead (Pb) pollution and identify the principal source(s) of environmental and human lead contamination in the area, lead concentrations and isotopic compositions in blood samples of children (ages 4–15 years) living within Villa de la Paz and Cedral, SLP, México, were determined by ICPQ-MS and compared to those of associated environmental samples (soil, tailings, and outdoor and indoor dust). Lead concentrations of soil, tailings and outdoor dust ranged between 73–2640, 1736–6700, 144–3550 mg Kg<sup>-1</sup>, respectively. Blood lead (PbB) concentrations levels in the children ranged between 2.6–35.9 µg dl<sup>-1</sup>, which is 3–15 times higher than the current average (1.9 µg dl<sup>-1</sup>) of children (ages 1–5 years) in the US. Lead isotopic ratios of the urban dust, soil and PbB actually are currently being measured.

**RP253 Assessing Estrogenic and Androgenic Activity of UV Filter Photoproducts** S. Jensen, K. Wammer, D. Martinovic, University of St. Thomas. Previous research suggests that some of the UV filters most commonly used as active ingredients in sunscreens may exhibit estrogenic or androgenic activity. When these UV filters are exposed to sunlight, they can break down into photoproducts, which are also potential endocrine

disruptors. For example, benzophenone, a once widely used sunscreen ingredient that does not have endocrine disruptor activity, breaks down into two photoproducts that have estrogenic activity. The goal of this project was to study UV filters for which information about endocrine activity of photoproducts was lacking. Octyl methoxycinnamate (OMC), a very commonly used UV filter, has been shown to exhibit androgenic activity both *in vitro* and *in vivo* at high concentrations. Another widely used sunscreen, padimate O (OD-PABA), has also been tested for both androgenic and estrogenic activity *in vitro* but has not been shown to express activity in either category. Here, several UV filters, including OMC and OD-PABA, were exposed to synthetic sunlight to generate photoproduct mixtures. The photoproduct mixtures were characterized using HPLC and LC-MS. Pure UV filters, photolyzed UV filter solutions containing a mixture of photoproducts, and one isolated photoproduct from the photolysis of OMC (4-methoxybenzaldehyde [4-MBA]) were screened for endocrine activity with two transcriptional assays. T47D-KBluc and MDA-MB-453 cells were used to assess estrogenic and androgenic activity, respectively. The endocrine activities of the samples were interpolated by the least-squares means procedure from a nonlinear sigmoidal dose response curve fit to the relative luminescence units of the estradiol/testosterone standards. OMC exhibited low-level estrogenic as well as androgenic activity. The photoproduct 4-MBA showed weak estrogenic and androgenic activity. OD-PABA, as previously suggested, does not exhibit androgenic activity whereas a mixture of its various photoproducts was found to have detectable activity. The estrogenic activity of OD-PABA's photoproducts has yet to be analyzed.

**RP254 Bioaccumulation of Emerging Contaminants in Edible Crops** K.C Hyland, A.C. Blaine, C.P. Higgins, Colorado School of Mines / Civil and Environmental Engineering. Anthropogenic organic contaminants present in wastewater which may persist through treatment processes pose a complex and relevant issue when treated wastewater, or reclaimed water, becomes a viable resource for agricultural irrigation or when wastewater-derived biosolids are land- applied in agricultural settings. The vast range of types of chemical contaminants resulting from human use includes pharmaceuticals, flame retardants, corrosion inhibitors, surfactants, plasticizers, and many others. The presence of many of these in reclaimed water and biosolids has been well documented. As a consequence, concerns have arisen about the potential bioaccumulation of trace- level organic contaminants into crops exposed to reclaimed water or biosolids. Previous studies have documented the potential for contaminant bioaccumulation of some pharmaceuticals, surfactants and antimicrobials into food crops. This study combined field-based sampling and controlled greenhouse experiments in order to examine the potential for bioaccumulation of a suite of chemicals commonly found in wastewater into selected edible crops irrigated with reclaimed water or grown in biosolids- amended soil. Results from this study have important implications with respect to the potential routes of trace-level organic contaminant exposure to humans.

**RP255 Evaluation of NDMA and precursors before and after advanced oxidation using a novel GCMSMS method** A. Kahl, University of Arizona / Department of Chemical and Environmental Engineering; S. Snyder, University of Arizona / Chemical and Environmental Engineering, University of Arizona / Chemical & Environmental Engineering. Nitrosamines are a group of chemicals potentially formed during water disinfection. Among them, the most well-known is nitrosodimethylamine (NDMA). NDMA is a model compound for nitrosamines and its genotoxicity is well known. The US EPA considers NDMA as a B2 chemical (probable human carcinogen). In addition to the potential health effects mentioned above, NDMA is also one of the 104 chemicals on the third contaminant candidate list of the US EPA. Thus, its concentration in drinking water could be regulated in the near future. Consequently, assessing the potential formation of NDMA in engineered and de facto water reuse scenarios is critical. Although several studies have been carried out on NDMA formation the formation mechanisms is not fully understood and probably some precursors are not yet identified. In particular, more studies are needed on the formation of NDMA during ozonation. Due to the lack of information on the precursors in the formation of NDMA, especially during ozonation, and to the occurrence of this probable human carcinogen during disinfection of water and wastewater, more research is needed in order to better understand the mechanism of NDMA formation during ozonation, what precursors play a key role and how to minimize and control NDMA in water. A series of experiments were conducted to assess NDMA concentrations in water both



before and after ozonation and advanced oxidation using a novel GC/MS/MS method for analysis. Data were then assessed to determine the degree of contribution of advanced treatment methods to NDMA formation and mined for precursor elucidation.

**RP256 Transport Potential of Perfluoroalkyl Substances (PFASs) at AFFF-impacted Sites: Batch Sorption and Initial 1-D Column Experiments** J. Sepulvado, Colorado School of Mines / Hydrologic Science & Engineering, Colorado School of Mines; C. Higgins, Colorado School of Mines / Civil & Environmental Engineering. The recent implementation of soil and drinking water regulatory guidance values for two perfluoroalkyl substances (PFASs), perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) by the U.S. Environmental Protection Agency (EPA), reflects growing concerns regarding the presence of these persistent and bioaccumulative chemicals in the environment. Because of their unique properties, PFASs have a wide variety of uses including food paper packaging products, stain repellants, nonstick coatings, and aqueous film-forming foams (AFFF). AFFF is used to extinguish hydrocarbon fuel fires by fire departments, the hydrocarbon industry, and the military. Concentrations of PFASs in ground-water have been measured in the ng/L – µg/L range at fire training facilities where AFFF was repeatedly used in training exercises. These sites may also be impacted by co-contaminants, including non-aqueous phase liquids (NAPLs) and other AFFF-components. Thus, the present work investigates the transport potential of 13 PFASs in the subsurface by evaluating sorptive behavior to various solid phases in systems both in the presence and absence of NAPL and other co-contaminants as well as under equilibrium and advective conditions. This study is the first to report on PFAS sorption over the concentration range applicable to AFFF-impacted sites. With the exception of short-chain PFASs, we observed isotherm nonlinearity and trends with PFAS chain length and organic carbon content ( $f_{oc}$ ) that are comparable to previous work. Competitive sorption between PFASs was observed in low  $f_{oc}$  solid phases. The addition of NAPL to the solid phase has the potential to increase sorption of PFASs in low- $f_{oc}$  solid phases; whereas sorption of PFASs may be decreased when NAPL is present in high- $f_{oc}$  sorbents. The impacts of NAPL depend on the sorbate and soil type, but are relatively independent of NAPL type. Preliminary column data suggest that PFAS transport will be chain-length dependent even under non-equilibrium conditions. Ground-water transport potential of PFASs at AFFF-impacted sites is expected to be highly dependent on the  $f_{oc}$  of the solid phase, PFAS chain length, and on the co-contaminants present.

**RP257 Understanding How Hydrophobic Organic Pollutants Distribute in Urban Runoff: Stormwater and Dry Weather Flow** M. Hsu, University of California, Los Angeles (UCLA) / Environmental Science and Engineering, UCLA; B. Schwegler, Walt Disney Imagineering / R&D; M. Stenstrom, University of California, Los Angeles (UCLA) / Civil and Environmental Engineering; M. Suffet, University of California, Los Angeles (UCLA) / Environmental Science and Engineering. Polycyclic aromatic hydrocarbons (PAHs), a group of the hydrophobic organic pollutants (HOPs), are mainly generated from incomplete combustion and commonly found in the urban runoff. PAHs have been targeted to control due to their prevalence, persistency and toxicity. Researches have estimated that urban runoff results in 14-36% total PAHs loading into aquatic ecosystems. However, most studies for PAHs in urban runoff have only studied the particulate phase and during the rain events. The pollution of HOPs, such as PAHs, in the dissolved phase of urban runoff has not been clarified. Dissolved organic matter (DOM) is ubiquitous in the aquatic environment and with different characteristics. DOM has the ability to associate with HOPs in natural waters like urban runoff. The association between HOPs and DOM affects the bioavailability of HOPs and their resulting toxicity. In other words, when DOM associates with HOPs to become a bound form, DOM-HOPs, in the dissolved phase, is not toxic to biota. On the other hand, the free portion of HOPs is the truly toxic part. In aquatic environment, knowing true free concentration of HOPs is necessary to effectively determine the bioavailable quantity of HOPs and minimize environmental hazards. This study used the PAH, perylene, as a probe for HOPs. Partition coefficients between DOM and perylene ( $K_{DOM}$ ) were measured by the fluorescence quenching method. Characteristics of DOM were measured for: 1) size distribution 2) polarity and 3) UV and fluorescent chemical functionality. The results showed that DOM characteristics show seasonal variations and there is a significant difference between dry weather runoff and storm events. Thus the relationship between DOM and HOPs in equilibrium with DOM-HOPs needs

definition on a seasonal basis. Specific UV Absorbance (SUVA) of bulk samples and  $UV_{254}$  within DOM in the 10k-5k molecular weight size fraction appear to primarily affect association between DOM and PAHs. The results can help understand the distribution of hydrophobic organic pollutants in the urban runoff in the dissolved phase and potential toxicity of free PAHs. The information can be used for reference for future regulations on the HOPs. The procedure developed in this study can also help optimize the Toxicity Identification Evaluation (TIE) process required in the National Pollutant Discharge Elimination System (NPDES) permit by relating the toxic free PAHs from the non-toxic bound DOM-PAH complex.

**RP258 Uptake of Cadmium and Selenium from Quantum Dots by *Eisenia andrei*** D.T. Stewart, K.O. Noguera, V. Lee, S. Banerjee, D.S. Aga, State University of New York-Buffalo / Chemistry. Engineered nanomaterials (ENM), including quantum dots (QD), are playing an increasingly important role in research and product development. As these materials are added to more products, their environmental effects will need to be assessed to ensure proper handling. Impacts of ENM on cell cultures have been extensively studied but whole organism studies are likely to be more relevant when elucidating environmental fate and toxicity. This work addresses the uptake behavior of cadmium selenide QD in an artificial soil environment by *E. andrei* earthworms. Worm samples, after one week and four weeks of exposure to QD, were digested and analyzed by inductively coupled plasma mass spectrometry. In addition, positive control samples consisting of worms exposed to cadmium nitrate and selenious acid for the same period of time were also analyzed. Worms had accumulated cadmium and selenium from the enriched soil over both time periods: 9.2 and 1.6 fold accumulation respectively after four weeks. The metal concentrations also increased with exposure time, indicating further uptake of metal ions might occur with longer exposure. The mole ratio of cadmium to selenium in the QD exposed worms ( $6.20 \pm 0.50$ ) is closer to the ratio seen in positive control worms ( $7.18 \pm 0.55$ ) than to the pure QD (1.8). These results suggest that the worms take up cadmium and selenium after QD disintegration in soil. Importantly, bioaccumulation of cadmium and selenium is reduced in the QD exposed worms compared to the positive control worms.

**RP259 MicroRNAs as Novel Toxicity Biomarker in Earthworms** P. Gong, SpecPro Inc. / Environmental Services, SpecPro Inc.; N. Barker, SpecPro Inc.; N. Wang, University of Southern Mississippi; E. Perkins, US Army Engineer Research and Development Center. MicroRNAs (miRNAs), an abundant new class of non-coding small RNA that average 22 nucleotides in length, have recently been discovered playing important roles in gene regulation and toxicity pathways. As key representatives of soil fauna, earthworms form the foundation of a healthy soil ecosystem and can serve as early bioindicators of chemical stressors such as explosive compounds resulting from military related activities. Our previous toxicogenomic studies have shown that sublethal levels of TNT and RDX affect expression of protein-coding messenger RNA (mRNA) genes which can be linked to neurological dysfunction, blood disorders, weakened immunity, or reproductive toxicity in *Eisenia fetida*. However, the precise molecular mechanisms of toxicities remain largely veiled due to the lack of knowledge and poor understanding of the highly complicated gene regulation on both spatial and temporal scales in *E. fetida*. In the current study, we discovered over 100 miRNAs and experimentally validated 31 of them, including one novel miRNA never being reported. Expression of miRNAs was affected by TNT or RDX as early as within 24 hr of exposure. TNT and RDX exhibited differential effects on miRNAs in the earthworm *E. fetida*. Computational prediction and bioinformatic annotation of target earthworm mRNA genes of identified earthworm miRNAs revealed biological pathways consistent with our previous studies. These results suggest that miRNA can serve as sensitive and rapid biomarkers for adverse effects in earthworms.

**RP260 Influence arsenic in the concentration elemental's in *Azolla filiculoides*** G. Sanchez Viveros, Universidad Veracruzana / Facultad de Ciencias agrícolas-Campus Xalapa; R. Ferrera Cerrato, Colegio de Postgraduados / Area de Microbiología del suelo, programa de Edafología; A. Alarcon, Colegio de Postgraduados; F. Reyes Zepeda, Instituto Tecnológico Superior de Tlaxiaco. This study determined the accumulation of arsenic in the fronds of *Azolla filiculoides* which had concentrations of P, S, Ca and Zn. The fern was exposed Yoshida's nutrient solutions contaminated with increased concentrations of sodium arsenate ( $0$  to  $30 \text{ mg mL}^{-1}$ ). After 96 h, the fronds were harvested and oven dried to estimate the bioaccumulation

factor (BCF) and to analyze element concentrations using X-Ray fluorescence (XRF) methods. The BCF significantly decreased as the concentrations of As increase in the nutrient solutions. *Azolla filiculoides* tolerated concentrations lower than 30 µg As mL<sup>-1</sup>. The accumulation of As in the fronds resulted in a significant increase on the content of P. This is one of the first reports about the influence of As on the growth and on the accumulation of As in *Azolla filiculoides*; in addition, the impact of this metalloid on the concentration of different elements in the fronds of this fern is reported.

**RP261 Exocytotic membrane proteins (SNAREs) in the rotifer *Brachionus calyciflorus* exposed to carbaryl and methyl parathion pesticides** I.A. Perez-Legaspi, Instituto Tecnológico de Boca del Río / División de Estudios de Posgrado e Investigación; R. Rico-Martínez, Universidad Autónoma de Aguascalientes / Departamento de Química; J. Quintanar, Universidad Autónoma de Aguascalientes / Departamento de Fisiología y Farmacología. The organophosphate and carbamate pesticides methyl-parathion and carbaryl have a common action mechanism; they inhibit acetylcholinesterase enzymes by blocking the transmission of nerve impulses. However, they can alter the expression of exocytotic membrane proteins (SNARE), by modifying the release of neurotransmitters and other substances. This study evaluated the adverse effects of the pesticides methyl-parathion and carbaryl on the expression of several SNARE proteins: Syntaxin-1, Syntaxin-4 and SNAP-23 in the freshwater rotifer *Brachionus calyciflorus*. Protein expression of these three proteins was analyzed before and after exposure to these two pesticides by Western blot and polyacrylamide gel electrophoresis. Analysis of protein expression was performed by densitometry of the bands through an image analysis system. The statistical analysis of the data was using analysis of variance of one-way ANOVA ( $p < 0.01$ ). The expression of Syntaxin-1, Syntaxin-4 and SNAP-23 proteins in the rotifer *B. calyciflorus* significantly decreases with increasing concentration of any of the two pesticides evaluated. This suggests that organophosphates and carbamates have adverse effects on the expression of membrane proteins of exocytosis by altering the recognition, docking and fusion of the presynaptic and vesicular membranes involved in exocytosis of neurotransmitters. They also modify the release of hormones and other proteins. Our results demonstrate that the neurotoxic effect of anticholinesterase pesticides influence the interaction of syntaxins and SNAP-25 and the proper assembly of the SNARE complex.

**RP262 Histological biomarker in oysters from Tampamachoco Lagoon, Mexico** I. Camarena, X. Guzmán-García, Universidad Autónoma Metropolitana; A. Botello, Universidad Nacional Autónoma de México; H. González-Marquez, Universidad Autónoma Metropolitana / Ciencias de la Salud – Área de Biología Celular y Molecular. Coastal lagoons in Mexico are impacted by urbanization. The use of biomarkers is appropriate to assess the health of the resident organisms in aquatic systems since it allows the extrapolation of results to the ecosystem. The oyster *Crassostrea virginica* was used as a bioindicator because of its sedentary and filtering life style. The oyster histopathology was studied to determine damage caused by pollution in target tissues. The study took place in Tampamachoco Lagoon an area contaminated with pesticides and hydrocarbons. We evaluated the oysters' health through the analyses of cells and tissues from their digestive gland. Organisms were collected during dry and rainy seasons. The digestive tubules were mainly in disintegration and reconstitution, meaning that food availability was very low in both seasons. Some mild to severe swelling was observed in the tubules (the homogeneity of the tissue was interrupted by holes). The coating epithelium did not show a clear stratification among its different layers making difficult to identify differences among and between layers. In some organisms brown cells associated with swelling and inflammation were observed, their presence could be explained by the finding of *Nematopsis* sp. parasites. Histological results showed damage at cell and tissue levels that could be caused by biological condition and/or pollution. Additional short and long assessments are needed since these organisms are exposed to contaminants during their life span.

**RP263 Morphological alterations in the freshwater rotifer *Brachionus calyciflorus* Pallas 1677 (Rotifera: Monogononta) caused by vinclozolin chronic exposure** J. Alvarado-Flores, Universidad Autónoma de Aguascalientes / Centro de Ciencias Básicas. Vinclozolin is a dicarboximide fungicide widely used on fruits, vegetables and wines, effective against *Botrytis cinerea*, *Sclerotinia sclerotiorum* and *Minilium* spp. In this study we characterized the effects of vinclozolin using a 4-day reproductive chronic assay with the

freshwater rotifer *Brachionus calyciflorus*. The assay included observations of several features of asexual and sexual reproduction. Our results indicate that vinclozolin: a) increase asexual and sexual reproduction, b) caused abnormal females and c) these abnormality are inherited by sexual and asexual reproduction. In an exposure range of 1.2 to 1.6 mg/L vinclozolin deforms the normal morphology in females at the end of 4 days. In fact, at 1.2 mg/L we found 3 abnormal females out of 457 total females (0.65% abnormalities). This percentage, although small, is consistent and reproducible. We increased the number of replicates and total females exposed to 1.2 mg/L of vinclozolin, and found 18 abnormal females out of 2868 total females (0.63% abnormality). In a range of 0.4 to 5.6 mg/L, vinclozolin exposure also caused the presence of abnormal females. At 1.2 mg/L vinclozolin also increased the size of vitellarium in normal females, and at 1.6 mg/L vinclozolin increased the size of males and eggs. Vinclozolin is an antagonist to the progesterone receptor and possibly affects the progesterone receptor in rotifers thus affecting several stages of asexual and sexual reproduction. Interestingly the abnormal females formed at 5.6 mg/L vinclozolin were capable of carry on normal mating behavior.

**RP264 Designing Quantitative Structure Activity Relationships (QSAR) to predict specific toxic endpoints in mammalian cell culture systems** S. Rawat, E. Bruce, Baylor University / Environmental Science and Biomedical Sciences. Polybrominated diphenyl ethers (PBDEs), a current-use flame retardant, have vast industrial application in products, such as plastics, building materials, electronics, textiles, etc. They are structurally similar to thyroid hormones that are responsible for regulating metabolism in the body. Therefore, PBDEs compete for the thyroid hormone binding receptors. This adversely affects thyroid hormone transport and metabolism/deactivation in the system (Meeker et al., 2009; Darnerud, 2008; Legler, 2008). Due to their potential threat to human health, this study aimed to design Quantitative Structure Activity Relationship (QSAR) models for predicting specific toxic endpoints, namely, cell viability and apoptosis. Human hepatocarcinoma (Hep G2) cell line exposed to PBDEs were used as a model system to evaluate cell viability using Janus Green dye and apoptosis using a caspase assay. The data collected from the experiments were used to create QSAR models using Genetic Function Approximation (GFA) method to predict the endpoints. QSAR modeling produces statistically robust and reliable models that reduce time and cost of experimentation and can further lead to more focused experimentation.

**RP265 Quantification of perfluorinated acid precursors in AFFF formulations and AFFF-contaminated groundwater and sediments** E. Houtz, University of California, Berkeley; D.L. Sedlak, University of California Berkeley / Department of Civil and Environmental Engineering. Aqueous film forming foams (AFFF) are complex mixtures of hydrocarbon and fluorocarbon surfactants that have been used by the DoD since the 1960s to extinguish fires. Within military bases, the groundwater and sediments beneath unlined fire-training areas are heavily contaminated with polyfluorinated and perfluorinated alkyl substances (PFASs) from the use of AFFF, including perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS). In addition to the perfluorinated acids, AFFF formulations contain related polyfluorinated compounds, i.e. precursors, that are transformed to the perfluorinated acids as terminal products of microbial and biological metabolism. There are more than twenty identified precursors to the perfluorinated acids found in various AFFF formulations and there are many possible intermediate products of these precursors' biological transformation; the direct measurement of all of these compounds in field samples is limited by lack of available standards and the difficulty of creating a single comprehensive analytical method for their analysis. In response to this challenge, a method was developed to indirectly determine the concentrations of potential precursor compounds. Samples were exposed to hydroxyl radicals generated in aqueous solutions and precursors were converted to measurable perfluorinated carboxylic acid products. This total oxidizable precursor (TOP) assay was adapted to measure precursor concentrations in dilute AFFF formulations and groundwater and sediment extracts taken from a contaminated fire-training area at a military base. Preliminary results demonstrate that TOP concentrations are significantly higher on sediments than in corresponding groundwater samples. Additionally, precursor compounds on sediments are measured at concentrations comparable to the perfluorinated acids. The ratios of TOP and perfluorinated acid concentrations measured in AFFF-solutions were used to provide insight into biotransformation of AFFF-derived precursors in field samples. A comparison of these

ratios between dilute-AFFF and AFFF-contaminated groundwater and sediment samples suggests that significant biotransformation of AFFF-derived precursors to terminal perfluorinated acids has occurred in the contaminated fire-training area. These findings indicate that AFFF-precursors may represent an ongoing source of perfluorinated acids at AFFF-contaminated military bases.

**RP266 A Dissolved / Particulate Baseline Study of PAH's (Poly-Aromatic Hydrocarbons) in Particulate Laden Water** M. Ebitson, D. Gallagher, Horizon Technology Inc. / Applications. The purpose of this investigation/method development was to establish a new extraction method using hexane as the extraction solvent for the determination of 209 PCB congeners in 116 chromatographic peaks in various ratios in aqueous samples which would allow for a detection range into the ng/L (PPT) range for 1L and 8L samples while processing the samples through the disk at over 100 mL/min. This was accomplished by optimizing a Solid Phase Extraction (SPE) method based on the Horizon Technology SPE-DEX® 4790 Automated Extractor System using a divinylbenzene (DVB) extraction disk and detection through a GC/ECD. The sample preparation step is an essential element of this method development, and as such, the advancement of an automated disk extraction has resulted in less solvent use, elimination of the solvent exchange step, reduced glassware use, faster extraction time with highly particulate samples, and more consistent and reproducible results. The results are excellent for all of the selected persistent organic polluting PCB congeners concentrated down to 1.0 mL on the DryVap®. If there was to be any loss of PCBs it would be during the extraction. The average recovery levels range from 95% for hexachlorobiphenyl (#169) to 112% for dichlorobiphenyl (#15.) from the 209 congeners. The average standard deviation for the entire data set is 4.3. The results of this testing illustrates the method detection limits (MDL) for two initial sample volumes. The results show that this methodology is able to achieve low MDLs of 0.97 ppt for 8-liter samples and 9.34 ppt for 1-liter samples for total PCB. A total of 116 chromatographic peaks were detected, containing 209 PCB congeners in various ratios. This allows an almost complete profile of environmentally occurring PCBs and shows that the SPE-DEX automated extractor can not only process samples at over 100 mL/min but can retain PCB's congeners at a low ppt level with a fast sample flow rate. This method shows that with tighter regulation and lower detection limits being required by regulating authority's analytical laboratories have to find improved methods of extraction and analysis. The challenge for any laboratory is meeting regulatory needs and wants of increased sample throughput, shorten turn around times and achieve reproducible results while providing lower detection limits. With this method based from Automated SPE by GC/ECD detection those requirements can be met.

**RP267 New Developments in Automated Solid Phase Extraction Methodology for Semi-Volatiles** M. Ebitson, Horizon Technology Inc. / Applications. In the laboratory industry, the most challenging methods for extracting water are for Semi-Volatiles. Semi-volatiles that are extracted by traditional techniques are laborious, time consuming and due to the complexity of the matrix can provide poor results if not handled properly. For solid phase extraction with 47mm disks it was questioned due to particulates, solids and the need to change pHs if it could even be extracted effectively. All those complications encountered can lead to frustrating suspensions which in turn lead to long sample process times during SPE. This paper will explain new developments in automated SPE technology, methodology and chemistry. These new developments not only provide a solution for processing troublesome water samples with an automated SPE extractor, but in fact provide an increase in recoveries for troublesome compounds that are experienced with traditional extraction techniques including past SPE methods. The following Horizon Technology instruments were used for this research, the SPE-DEX 4790 Automated Extractor and the DryVap Concentrator System. The analysis will be conducted with an Agilent 6890 GC with an Agilent 5973 mass selective detector. The results will include a list of well over 100 semi-volatile compounds, of which will include many compounds that are known to have recovery issues during the extraction and concentration process. From the troublesome compounds the results illustrate how they were increased to levels well above the quality control acceptance limits. These new developments will provide laboratories with an efficient option for extracting different water matrix types without sacrificing results.

**RP268 Processing Highly Particulate Laden Samples Using Automated SPE Extractors** D. Gallagher, Horizon Technology Inc. / Applications. Oftentimes, aqueous samples collected contain varying amounts of suspended solids or sediment strictly due to either the source of the water being sampled or improper sampling techniques. In any circumstance, samples with high amounts of particulates or sediment have proven challenging to extract using EPA Method 3535: Solid Phase Extraction (SPE). The two options available for SPE are to either use a disk or a cartridge. Processing a high sediment sample with a SPE cartridge will result in instant clogging of the cartridge and failure to extract the "whole sample". A standard 47 mm SPE disk provides many advantages over SPE cartridges. SPE disks can process a wide range of particulate laden samples, but the amount or size of the particulates may sometimes result in longer sample processing times. An alternative is to use a 100 mm SPE disk which has nearly four times the surface area of a 47 mm disk and will process a particulate laden sample at a faster flow rate. However, larger disk sizes require more sorbent and can be very expensive. It would be more cost-effective to use an inexpensive 90 mm prefilter with a 47 mm SPE disk. This poster will demonstrate the development of various extraction methods for a specialized disk holder suited for automated SPE extractors. Multiple sets of data will be presented including data from samples extracted for EPA Method 8270D for Semi-Volatiles and EPA Method 608 for Poly-Chlorinated Biphenyls and Organochlorine Pesticides.

**RP269 Soil Microbial Community Diversity Analyses to Examine Resistance and Resilience of Soils to Base Oils in Land-Based Drilling Fluids** D.L. Carr, Texas Tech University / Biological Sciences; T.A. Anderson, Texas Tech University / Environmental Toxicology; S. Hughes, Shell Health – Americas. Microbial diversity and function in soil provides nutrients necessary for primary productivity in an ecosystem. Theoretically, high diversity imparts high ecosystem resiliency and recovery following a disturbance. Soil microbial community diversity was examined in two soils with next-generation deep sequencing technology to assess the soil microbial community response to four base oils used in land-based drilling fluids after 90 days of weathering at 10°C and 30°C. The base oils tested were Diesel, low toxicity mineral oil (LTMO), and two different gas-to-liquids (GTL) base oils. In general, the silt loam (Harlan County, NE) control soil responded with a decreased soil microbial Shannon Diversity Index (H') at 30°C, while the sandy loam (Terry County, TX) control soil exhibited a decrease in microbial community H' at 10°C. Richness was not greatly affected by temperature in either soil compared. Incubation temperature had an effect on community species identity in both soils; 44% of initial species were observed at 10°C and 4% at 30°C after 90 days of weathering. There was a trend of reduced H' when warm climate sandy loam soil was incubated at 10°C and cooler climate silt loam soil was incubated at 30°C. Soil microbial diversity declined 94% and 95% with Diesel exposure in sandy loam soil at 10°C and in silt loam soil at 30°C respectively. Silt loam soil incubations with base oils at 30°C resulted in a distinct community discomposition compared to the control with greater than 50% of the microbial communities consisting of a single species or microbial family. In comparison, sandy loam soil domination by single species or family occurred only in Diesel and LTMO treatments at 10°C. Even when base oils did not adversely impact microbial diversity, there was community discomposition observed in both soil types. The ability to choose base oils based on maintaining soil microbial diversity may potentially reduce costs of post-drilling site remediation. However, the impacts of discomposition or reduced diversity on soil microbial community function are not fully known and would be of interest in follow-up studies. This study suggests that LTMO and GTL base oils have less adverse effects on soil microbial community diversity in most situations than the conventional base oil Diesel.

**RP270 Temporal effects of releases on fish tissue concentrations – implications to achieving net risk reduction goals using different cleanup approaches** J. Stern, King County-DNRP / Dept. of Natural Resources, King County Dept of Natural Resources; B. Nairn, King County Dept of Natural Resources. Dredging has been the primary remedy to address contaminated sediments. But recent studies have demonstrated dredging releases to the environment can be as significant as long-term releases from material left in place. Calibrated sediment transport / recovery potential and bioaccumulation modeling paired with empirical data from a highly urbanized salt-wedge estuary on Puget Sound provides the opportunity to investigate differences in effects to the food web predicted to occur from



different cleanup approaches. Detailed contaminant and fate and transport modeling using EFDC was conducted to investigate how dredge-generated contaminated sediment residuals, along with associated releases of contaminants into the water column, can temporarily increase the bioavailability and compare that to remedies that leave contaminants in place but are an ongoing source to the food web. The data set includes fish tissue concentrations of PCBs that spiked by a factor of three to five and then returned to previous levels. Several sediment remedial actions occurred in the estuary six to nine months before the sampling that exhibited the high tissue concentrations. Comparison of measured tissue level reductions over time to bioaccumulation modeled responses also provides information on the persistence of dredging-related releases in the system. Combined with results of recent national studies, relative contributions of sediment-associated and porewater contaminants released by the various remedies can be predicted and the temporal responses of the food web can be estimated. Combined with information on consumption rates of various at-risk populations, results can help inform evaluations of short-term increase and longer term reduction in risk to determine the timing of achieving net risk reduction from sediment cleanup options. Implications for cleanup decisions and the approach to remediation for such sites are discussed.

**RP271 Quantification method development for determination of parabens in human urine by LC-MS/MS** S. Lee, KFDA, Korea Food and Drug Administration / Health effect analysis team, National Institute of Food and Drug Safety Evaluation, KFDA; E. Son, J. Kang, H. Nam, H. Jeong, H. Lee, M. Shin, S. Kim, G. Rhee, Korea Food and Drug Administration / Health effect analysis team, National Institute of Food and Drug Safety Evaluation. Parabens, esters of p-hydroxybenzoic acid widely used as antimicrobial preservatives in cosmetic products, pharmaceuticals and food and beverage processing. However, they have been revealed as weak estrogenic activity of some parabens through in vivo and in vitro studies. Because such widespread use has raised concerns about the potential human health risks associated with the exposure to parabens, we have developed an analysis method in human urine, using solid phase extraction with reverse phase cartridge and HPLC coupled with triple quadrupole mass spectrometry in Multiple Reaction Monitoring mode to measure the urinary concentrations of methyl, ethyl, propyl and butyl parabens. Also this method showed good specificity, linearity ( $R^2 > 0.999$ ), accuracy (92.2 - 112.4%), precision (0.9 - 9.6% as CV), and recovery (95.7 - 102.0%). These LOQs on 4 parabens were 1.0, 0.5, 0.2 and 0.5 ng/mL, respectively. And it could be used for quick and accurate analyses of large numbers of human samples in epidemiologic studies to assess the prevalence of human exposure to parabens.

**RP272 Toxicity of Untreated and Ozone-treated Oil Sands Process-Affected Water to Early Life Stages of the Fathead Minnow (*Pimephales promelas*)** S. Patterson, University of Saskatchewan / Toxicology Centre, Toxicology centre, University of Saskatchewan; Y. He, M. Hecker, University of Saskatchewan / Toxicology Centre; J.W. Martin, University of Alberta / Department of Laboratory Medicine and Pathology, Division of Analytical and Environmental Chemistry; M.G. El-Din, University of Alberta / Department of Civil and Environmental Engineering; J.P. Giesy, University of Saskatchewan / Biomedical Veterinary Sciences & Toxicology Centre, Michigan State University / Department of Animal Science, University of Saskatchewan / Toxicology Centre; S.B. Wiseman, University of Saskatchewan / Toxicology Centre, University of Saskatchewan / Post-Doctoral Fellow. The Alberta oil sands are the world's second largest reserve of petroleum. There is growing concern regarding the environmental impacts of the increasing volumes of oil sands process-affected water (OSPW) being produced. OSPW is acutely and chronically toxic to fish and other aquatic organisms, but the mechanisms of toxicity are poorly understood. Early life stages of fathead minnows exposed to OSPW have a greater incidence of hemorrhaging, premature hatching, pericardial edema, and spinal malformations. The toxicity of OSPW is thought to be mostly attributable to water soluble organic compounds, in particular naphthenic acids (NAs). Ozonation of OSPW might be an effective method for remediation by reducing concentrations of dissolved organic compounds, including NAs. This study examined the effects of untreated and ozone-treated OSPW (O3-OSPW) on embryos of fathead minnows. To elucidate the potential mechanism(s) of toxicity, the transcript abundance of genes involved in biotransformation of xenobiotics, response to oxidative stress, and regulation of apoptosis were quantified. Embryos exposed to OSPW had significantly greater transcript abundance of CYP3A, GST, SOD, Casp9 and ApoEn by  $2.35 \pm 0.34$ ,

$2.15 \pm 0.26$ ,  $3.08 \pm 0.74$ ,  $3.26 \pm 0.57$  and  $2.38 \pm 0.25$ -fold, respectively, than those exposed to control waters. This indicates that exposure to OSPW might cause oxidative stress and apoptosis, leading to the development of malformations. None of the effects observed in the embryos exposed to OSPW were observed in those exposed to O3-OSPW. The results suggest that the dissolved organic fraction of OSPW impairs development of embryos of fathead minnows through oxidative stress and apoptosis.

**RP273 A field-based approach for assessing the impact of paper pulp mill effluent on the metabolite profile of fathead minnows (*Pimephales promelas*)** J.M. Davis, U.S. EPA, ORISE / National Exposure Research Laboratory, U.S. EPA; T. Collette, D.R. Ekman, US EPA / National Exposure Research Laboratory; G.T. Ankley, US EPA / Office of Research and Development, National Health and Environmental Effects Research Laboratory, Mid-Continent Ecology Division, U.S. EPA / National Health and Environmental Effects Research Laboratory; J. Cavallin, U.S. EPA, ORISE / National Health and Environmental Effects Research Laboratory; K. Jensen, M. Kahl, U.S. EPA / National Health and Environmental Effects Research Laboratory; D.L. Villeneuve, U.S. EPA / National Health and Environmental Effects Research Laboratory, U.S. EPA. Although evidence indicates that exposure to effluent from paper pulp mills (PME) can alter the body condition, secondary sexual characteristics, and reproductive success of aquatic organisms, there is currently little understanding of the biochemical mechanisms for these effects (e.g., changes in endogenous metabolites). Here, we present results from a field-based study that assessed PME exposure on the polar metabolite profile of fathead minnows (*Pimephales promelas*) during a temporary shutdown of a kraft pulp mill. Male and female fathead minnows were deployed in cages for 48-hrs during three separate time periods (pre-pulp mill shutdown, during pulp mill shutdown, and after the pulp mill was restarted) at two locations in the Duluth-Superior Harbor of Lake Superior, USA (10- and 200-m from a waste-water treatment discharge that is typically around 45% PME). Additional fish were exposed to pristine Lake Superior water for 48-hrs in flow-through aquaria under lab conditions. We then used  $^1\text{H-NMR}$  spectroscopy to measure polar metabolites in livers and subsequently applied principal component analysis (PCA) to compare field-deployed and lab-control fish that were collected during the same time period. Through this and other comparisons, we assessed the relative impacts of PME exposure on the metabolite profile of liver tissue. Results from the PCA scores plots indicated that the metabolite profile of male fathead minnows at both field locations were significantly different from lab-control males prior to the pulp mill shutdown. However, metabolite profiles of field-deployed males were not statistically different from the lab-control males during the pulp mill shutdown; differences became statistically significant when the pulp mill was subsequently restarted. Females exhibited little difference in their metabolite profiles during the sample periods; thus, effects of PME exposure appeared sex-specific. This combination of evidence suggests that exposure to PME may alter the metabolite profile of certain aquatic organisms, but that impacts can attenuate once exposure ceases. Moreover, we demonstrate the potential utility of field-based metabolomic studies for biologically-based exposure monitoring and for assessing likely modes-of-action of PME exposure on aquatic biota.

**RP274 Metal content and physicochemical parameters of Cuitzeo Lake sediments as indicators of contaminant human activities** C. Ponce de Leon, Facultad de Ciencias, UNAM / Biology; S. Cram, Universidad Nacional Autonoma de Mexico / Instituto de Geografia; I. Sommer, Instituto de Geografia, UNAM; P. Fernandez, IDAEA-CSIC / Environmental Chemistry; P. Fernandez-Lomelin, Instituto de Geografia, UNAM / Geografia Fisica; M. Hernandez, Facultad de Ciencias, UNAM / Biology. Cuitzeo Lake is the name of a body of water that lies between the states of Michoacan and Guanajuato; it is second in size in Mexico and it is one of the most representative basins of central Mexico with 3977 square kilometers. Its water comes mainly through the rivers Grande de Morelia, Queréndaro and some small streams. This lake is of great importance for the region as it helps regulate the climate of the basin; also it is the livelihood and habitat of various species of plants and animals such as herons, gulls, multiple reptiles, abalone, sedismundo, maruchero shrimp, the Canadian duck, etc. The economy of thousands of families depend on this lake: local fishermen draw minnows, crappie, frog, and tule mosquito; farmers from neighboring areas take advantage of the lake waters to irrigate crops of maize, sorghum, oats, wheat and vegetables. In recent years this lake was about to disappear due to the sediment and the small amount of water it receives from its tributaries.

The average depth of this lake is a little less than 2 meters. Due to this small water column, the lake sediments have become the sink of a variety of contaminants. In the present study a net sampling of the sediments from the perennial section of Cuitzeo lake was done. The sediments were analyzed for their metal content and physicochemical parameters. Small lake fish and plants were also sampled and metal content determined. In general terms, the values of the parameters differentiate three zones: a) the SE zone with contributions from the irrigation district and municipal wastewaters, b) the NE riverside which seems to be the least influenced by human activities and c) the SW area with atypical high concentrations of potentially toxic metals. The geostatistical maps of Fe and Al showed a geogenic distribution, while Ti, As and Zn although they could be modeled as geogenic origin, they showed certain sites with atypical high concentrations probably due to anthropogenic inputs. Additionally, the geostatistical maps for the organic matter content clearly showed the environmental impact of livestock activities. Due to the scale of the sampling, no geostatistical map were feasible for Pb, Cu, Ni and Cd but their special distribution suggests anthropic origin from the Federal Highway No. 43 and the Morelia-Salamanca highway that cross Cuitzeo lake. The metal concentration determined in the plants and small fish (charal) from the lake, did not show significant metal accumulation.

#### RP275 Attenuation of emerging contaminants in the Critical Zone

A. Conine, Skidmore College / Department of Biology; R. Maxwell, L. Abrell, J. Chorover, University of Arizona / Department of Soil, Water, and Environmental Science. The Earth's critical zone (CZ) is the near surface terrestrial environment where interactions involving rock, water, soil, and air occur. A number of important interfacial chemistry processes and reactions occur in the CZ that control the transport and cycling of a group of organic compounds known as "emerging contaminants" that includes endocrine disrupting compounds (EDCs) and pharmaceuticals and personal care products (PPCPs). These organic pollutants derive from everyday products including medicines, artificial sweeteners, and industrial chemicals and have been detected in water sources throughout the United States. Waste water treatment plants (WWTPs) are only partially effective at removing EDC/PPCPs which results in their release into the environment. In this project, we examined whether EDC/PPCPs are attenuated in the CZ by studying the extent to which their concentrations decrease with distance from a WWTP effluent spray field. Research was conducted in the Oracle Ridge site in the Santa Catalina Mountains Critical Zone Observatory (SCM-CZO, Southern Arizona). WWTP samples were analyzed directly, as were water samples taken from three sites progressively downstream from the spray field. Analytes were pre-concentrated using solid phase extraction prior to analysis with UPLC-MS/MS. The percent of analyte attenuated between the WWTP and the upper stream site varied between salicylic acid (34%) and sulfamethoxazole and estrone (100%), indicating that processes beyond freshwater dilution, such as sorption and biodegradation, are taking place in the CZ between the WWTP and the upper stream site.

#### RP276 Investigation into toxicity of chemical mixtures involving organophosphate pesticides: malathion and diazinon toxicity to *Daphnia magna*

A.H MacLeod, Western Washington University / Department of Environmental Sciences. Freshwater habitats in urban and agricultural regions are commonly found to contain chemical mixtures from several non-point runoff sources. The suburbanization of land use in close proximity to agricultural regions allows contaminants applied to be transported into the nearest waterway. The common over-application of pesticides by landscaping and agricultural practices causes excess toxicant to be washed away with runoff. Recent studies on mixture toxicity have concluded that single toxicant risk assessments may be underestimating toxicity of certain pesticides to aquatic organisms when mixing in aquatic environments. To manage the implicit risk it is critical to understand how individual chemical toxicity mixtures can affect the survival and behavior of non-target organisms. Organophosphate pesticides inhibit the activity of acetylcholinesterase (AChE) and can generate effects that can interfere with freshwater communities by alteration of lower trophic levels. Due to reported synergistic toxicity of organophosphate mixtures, it is critical to assess mixture toxicity. In this experiment 48-hour acute *Daphnia magna* toxicity tests were conducted using >98% analytical grade malathion and >98% analytical grade diazinon. The chemicals were tested at the following concentration ranges: malathion 0.70 – 11.20 µg/L and diazinon 0.37 – 6.00 µg/L. A mixture of malathion and diazinon also were tested to evaluate the potential additive, synergistic,

or antagonistic toxicity: malathion 0.70 – 11.20 µg/L in the presence of 0.75 µg/L of diazinon. The mortality results indicated potential antagonism as determined by probit nonlinear regression.

#### RP277 P-glycoprotein (P-gp) in the monogonont rotifer, *Brachionus koreanus*: molecular characterization and expression in response to pharmaceuticals

B. Kim, Hanyang University / Dept of Chemistry; J. Rhee, C. Jeong, Hanyang University; J. Lee, Hanyang University / Dept of Chemistry, Hanyang University Graduate School / Dept of Chemistry. P-glycoprotein is involved in the efflux of diverse chemicals, including hydrophobic compounds and pharmaceuticals as a first line of defense. Here, we firstly identified and characterized the P-gp (Bk-P-gp) gene in the rotifer, *Brachionus koreanus*. Bk-P-gp was highly conserved in genomic organization compared to the human P-gp gene. Messenger RNA expression of Bk-P-gp revealed that it would be regulated by temperature change via 14 heat shock response elements in its promoter region. Bk-P-gp showed a high similarity of motifs/domains compared to those of vertebrates in its amino acid sequences. To check whether Bk-P-gp would be inducible, we exposed *B. koreanus* to six pharmaceuticals including antibiotics for use in aquaculture and observed dose- and time-dependency on transcripts of Bk-P-gp for 24h over a wide range of concentration. Efflux assay and membrane topology supported its conserved function for transportation of a number of chemicals upon cellular damage. To reveal the effect of pharmaceuticals on the rotifer, we measured survival rate and population growth rate after exposure to six pharmaceuticals. In an acute toxicity test, both NOEC and LC<sub>50</sub> values for all the pharmaceuticals were high for 24 h. ATP, CBZ, SMX, and TMP markedly inhibited the population growth of *B. koreanus* after exposure up to 100 mg/L for 10 days. In this paper, we demonstrated that various pharmaceuticals can retard growth rate with up-regulation of the P-gp gene as a cellular defense system. This finding provides a better understanding of molecular mechanisms involved in pharmaceutical-mediated cellular damage in *B. koreanus*.

#### RP278 Quantifying Particle Number of Gold Nanoparticles by Multi-Method Approach

A. Prasad, University of Birmingham / Geography and Environmental Sciences; J. Lead, University of Birmingham / School of Geography, Earth and Environmental Science; M. Baalousha, University of Birmingham / Environmental health and risk management. According to the EU SCENIHR [1], certain physico-chemical properties of relevant nanoparticles are anticipated to have major impacts on their behaviour in the environment. Of particular relevance to this research, the appropriate metrics for characterising nanoparticles concentration as it is recognised, that the mass concentration used for conventional chemicals may be inappropriate [2]. Therefore, it is requisite to identify the most appropriate metrics and methodology for the measurement and characterisation of nanoparticles. The appropriate metrics to use in nanoparticle studies is an important policy issue of the 19 research objectives produced by the UK government (DEFRA) [3]. PVP and citrate capped gold nanoparticles were synthesized in-house and comparative study is performed to quantify the accurate particle number concentration under different dilutions. For the comparative studies, a wide range of microscopic analyses were carried out, which are: transmission electron microscopy (JEOL 1200 and Philips TECNAI F20), scanning transmission electron microscopy (STEM), scanning electron microscopy (SEM), atomic force microscopy (AFM) and nanoparticle tracking analysis (NTA). Instead of mass concentration, particle numbers were counted and compared using the above techniques in order to obtain consistent results. In future, these results were compared with a mathematical model. This will lead to, 'reference' NP suspensions of known particle number to be produced which could be made available for toxicological studies.

#### RP279 PBDE content in leachates and sludge from landfills in Mexico

I. Gavilan, E. Santos, A. Gavilan, Facultad de Química – UNAM. Brominated flame retardants are chemicals of increasing interest to scientists, government agencies and the public. These have been used in a variety of manufactured products, including foam cushioning used in furniture, and plastic housings for televisions and computers. Currently a large number of goods manufactured or imported to Mexico have in their formulation an unknown percentage of PBDEs and other brominated flame retardants; in addition, the environmental fate of these substances is unknown, and several releases from landfills might represent a potential risk to human health and the environment. This study analyzed the PBDE content in leachate

and sludge from two landfills in Mexico using Gas Chromatography / Mass Spectrometry. PBDE analysis included: BDE-17, BDE-47, BDE-49, BDE-66, BDE-85, BDE-99, BDE-100, BDE-101, BDE-138, BDE-153, BDE-154, BDE-183, BDE-190, BDE-203, and BDE-209. PBDE concentration in leachates ranged from 0.2-37 µg/L. PBDE concentration in sludge ranged from 75-425 ng/g. Specific concerns were raised of open burning since PBDE emissions might take place. On the other hand, specific activities should be developed to characterize the capacity of unofficial dumps to contain PBDEs through the analysis of leachates and biosolids, as well as air emission during regular operation and when burning activities are carried out.

**RP280 The Roles of Cyclin D1 and CDK4 in perfluorooctane sulfonate –induced apoptosis of cerebellar granule cells** Y. Lee, Catholic University of Daegu / Pharmacology/Toxicology; J. Yang, Catholic University of Daegu / Department of pharmacology and toxicology. Perfluoroalkyl compounds (PFCs) are widespread environmental pollutants and have been shown to affect fetal growth. Perfluorooctane sulfonate (PFOS) is a prominent member of PFCs and the perinatal exposure to PFOS elicits developmental neurotoxicity. We have recently reported that PFOS induced apoptosis of developing neurons in ERK-dependent manner. In this study, we examined the involvement of cell cycle proteins in PFOS –induced apoptosis of cerebellar granule neurons (CGCs) to further understand the underlying mechanisms. CGCs isolated from 7-day old rats were maintained in culture for additional 7 days to mimic the brain growth spurt period conditions. Then, cells were treated with PFOS for different times. PFOS exposure for 24 h resulted in caspase-3 activation and nuclear condensation and fragmentation, which were selectively inhibited by pretreatment with PD98059, an inhibitor of ERK pathway. Western blot analysis showed that PFOS increased cyclin-dependent kinase (CDK) 4, cyclin D1 and cyclin E which are known to be involved in G<sub>0</sub>/G<sub>1</sub> phase. The increases in CDK4 and cyclin D1 were blocked by PD98059. Taken together, our results indicated that PFOS induces apoptosis of cerebellar granule cells through ERK-dependent pathway and cell cycle modulators are downstream molecules affected by ERK.

**RP281 The Use of Additional Analytical Sampling Time Points Can Have a Significant Effect on Calculated Toxicity Values for Algal Growth Inhibition Tests** C. Mead, Harlan Laboratories Ltd / Ecotoxicology Dept.; H. Vryenhoef, J. Handley, Harlan Laboratories Ltd. The Algal Growth Inhibition Test (OECD 201) is by design a static test and hence in cases where the chemical of interest is unstable over the test period, modifications to the dosing regime such as semi-static or continuous test media renewal to maintain near nominal test concentrations cannot be used. As such under normal sampling schedules significant declines in measured test concentrations may occur over the test period which can result in lower than expected toxicity values. The inclusion of additional sampling points at intermediate times throughout the test period can better describe the decline in test item concentrations. Calculation of endpoints based on such analyses can have a significant effect on the calculated toxicity values and subsequent classification of the test item.

**RP282 Developing otolith microchemistry to determine fish residency, baseline geology and fish foraging patterns** V.P. Palace, Department of Fisheries & Oceans Canada, Stantec Consultants; L. Friedrich, Department of Fisheries and Oceans Canada; N. Halden, University of Manitoba / Geological Sciences. Otoliths, the inner ear bones in fish, have long been used as the most reliable means for ageing fish. Increasingly, microchemical analysis of trace elements in their metabolically stable matrix have been employed to make inferences about residence patterns, exposure to natural or anthropogenically elevated metal(oid) concentrations and foraging and migratory patterns. Microchemical analysis of the growth rings is typically accomplished by laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS), and divalent cations, which substitute for calcium in the otolith bone structure (eg. Zn, Mn, Mg, and Sr), are most commonly determined. However, elements that can be incorporated in the mineral and protein matrix of the otolith, including Se and S have also been reported. Selenium is of particular interest because of well documented, elevated exposure downstream from coal and uranium mines, and because of its potential to act as a reproductive toxicant in vertebrates that lay eggs. Maternal deposition of Se in eggs and assimilation by the developing embryo is the vector of most relevance. Using LA-ICP-MS, we have determined Se in growth zones of otoliths from several freshwater fish species from various locations.

By temporally resolving Se in the otolith and comparing these results with concentrations of Se in ovaries, relationships between lifelong exposure and discreet reproductive events can be explored. Moreover, important data gaps for the use of otoliths to retrospectively construct Se-exposure histories and potential reproductive impacts can be identified.





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